PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)

FOLLOW-UP OBSERVATIONAL STUDY II

PROTOCOL

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PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY II PROTOCOL
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PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY II PROTOCOL

CHAPTER 1

BACKGROUND AND STUDY RATIONALE

1.1 OVERVIEW

The Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG) was designed as a Phase III, two-year study treatment, double-blind, randomized placebo-controlled trial including 200 subjects with sickle cell anemia (SCA) at 14 clinical centers. The final subject (N=193) was randomized into the BABY HUG Treatment Study in September 2007. At the end of their child's two years of study treatment, all parents/guardians were offered the opportunity for their child to be treated with open-label hydroxyurea (HU) therapy, without regard to and without knowledge of their child's randomized treatment assignment. When originally consenting to participation in BABY HUG, parents/guardians were told explicitly of the investigators' intention to request permission to follow their child for many years, to evaluate possible long-term effects of treatment.

The BABY HUG Follow-up Study I was an observational study to provide structured monitoring of the subjects enrolled in the BABY HUG Treatment Study, to initiate characterization of the long-term toxicities and unexpected risks (if any) associated with treatment with hydroxyurea at an early age, and any potential benefits of therapy. There were 163 subjects from BABY HUG, treated for at least 18 months on the treatment study (average age was 4.8 years at the end of the treatment study) subsequently enrolled in the first Follow-Up Study (designated FUS I). Of those, 127 participated in the active protocol and 36 in the passive protocol for follow-up (for details on "active" vs. "passive" protocols, see 3-4. Informed Consent Template on page 3-4). . At consent to FUS I, 133 subjects were being treated with open-label HU. Approximately half of the 133 subjects received HU in the BABY HUG Treatment study and half received placebo. Of the 133 open-label HU subjects, 113

participated in the active arm and 20 in the passive arm of FUS I. Longitudinal comparisons between these two groups may provide important information regarding the timing of initiation of HU. All subjects in the study consented to periodic reporting of clinically obtained information including growth parameters, blood test results, transcranial Doppler ultrasound (TCD) or other clinically obtained routine "screening" studies, and details of sickle cell anemia related hospitalizations and health events. Collected blood specimens provided samples for surrogate markers of toxicity and clinical efficacy, such as measures of renal and spleen function and markers of DNA damage. The 127 subjects in the "active" group of BH FUS I were to be reassessed two years after exit from the BABY HUG Study, using age-appropriate neuropsychological testing, radionuclide liver/spleen scans, abdominal ultrasonography and central laboratory testing.

Follow-Up Study II (designated FUS II) is an observational study to collect data on enhanced neuropsychological, brain, cardiac, and pulmonary evaluations for this very well characterized cohort of subjects. This unique population facilitates a follow-up study of this magnitude. Measures of spleen and renal function and markers of DNA damage will continue to be collected. Assessment of other target organs in sickle cell anemia including pulmonary and cardiac function will be performed in addition to evaluation of developmental aspects of SCA and potential HU toxicity.

Data collected in Follow-Up Study II will be descriptively analyzed according to the original treatment assignment (HU versus placebo), and the subsequent independent decision by families concerning use of open-label HU in FUS I. Data collected in the passive follow-up portion of this study will determine whether early hydroxyurea treatment is associated with long-term toxicities and provide limited data regarding long-term efficacy. Data collected in the active follow-up portion of this study will identify long-term effects on organ dysfunction, and determine if duration of treatment and age of initiation (earlier vs. later) affect hydroxyurea's efficacy and toxicity. Information to be obtained from this follow-up study is vitally important to understanding

the risks and benefits of early treatment, and ultimately for creation of an optimal paradigm for hydroxyurea therapy in young children with SCA.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)

FOLLOW-UP OBSERVATIONAL STUDY II PROTOCOL

CHAPTER 2

OBJECTIVES AND DESIGN OF THE STUDY

2.1 INTRODUCTION

The purpose of this observational study is to perform clinical follow-up of subjects enrolled in the original Pediatric Hydroxyurea Phase III (BABY HUG) Clinical Trial through at least the first decade of life following completion of their period of randomized study treatment. This long-term follow-up study was envisioned at the conception of the BABY HUG Treatment Study, and parents/guardians as well as local Institutional Review Boards (IRB) were made aware of the Investigators' intent to follow the subjects in the consent forms obtained during the BABY HUG Treatment Study enrollment period. The overall goals of the BABY HUG Follow-Up Study II are to define more accurately the long-term risks and benefits of early HU treatment and to evaluate organ function, growth and psychosocial development, and the predictive value of biomarkers. All of this information is needed to determine the potential benefits or possible risks of HU intervention early in the life of subjects with SCA and provide information pertaining to possible practice guidelines for the first decade of life.

Long-term follow-up of BABY HUG subjects treated with HU at an early age is critical for several reasons. First, certain HU toxicities identified during the BABY HUG Treatment Study may persist during the follow-up period, while other toxicities may decline in incidence or severity, and still other new toxicities may arise. The profile of these events may also be different in subjects starting HU treatment at 9 to 18 months of age (in the randomized treatment study) as opposed to subjects starting treatment at 33 months of age or later (by parent or guardian choice during the open-label Follow-Up Study I). Serious toxicities, particularly those concerning growth and development, could become evident only years after initial HU exposure

and treatment. Subjects not treated with HU during the randomized treatment study, or at all, represent an important comparison group and will be followed in the same manner.

Previous studies have provided limited assessment of late toxicities associated with HU therapy in a small number of young subjects with SCA. In the HUSOFT extension study (Wang et al, 2001; Hankins et al, 2005), 17 infants (age 7 to 25 months) completed four years of treatment with HU and eleven completed six years. Hoppe (Hoppe et al, 2000) described eight subjects with a mean age of 3.7 years (range 2 to 5 years) treated with HU for an average of 2.6 years. While no unique adverse side effects and no apparent growth toxicities were reported in these two small series involving open-label HU treatment, longer follow-up on larger groups is required to support evidence-based decision making. Long-term follow-up of the subjects enrolled in the BABY HUG Treatment Study will provide a critical and unparalleled opportunity to evaluate long-term toxicity profiles associated with early HU exposure in a large number of well-characterized very young children with SCA and to specifically assess the potential spectrum and severity of growth, development, hematological and organ toxicities. Ongoing concern about long-term effects on growth and development, as well as data regarding the possible mutagenicity/carcinogenicity of HU, can only be addressed in this type of long-term follow-up study.

Secondly, in conducting Follow-Up Study II, it is important to document any long-term clinical and laboratory effects associated with HU therapy in this young age group. Clearly toxicities must be identified, but benefits identified during the BABY HUG Treatment Study may persist during the follow-up period, others may decline in magnitude, and still other new benefits may arise. Hematological effectiveness of HU therapy in terms of maintaining elevated levels of HbF was shown in HUSOFT, but confirmation of this benefit and its persistence with long-term treatment in a larger group of placebo-controlled subjects is needed. While it would be ideal to prospectively assess the beneficial effects of HU therapy in preventing or ameliorating chronic organ damage with “gold-standard” testing at several times over many years, simple less

invasive measures of organ damage and function have been shown to be useful in the BABY HUG Treatment Study. Accordingly, we will collect blood and urine samples at study entry and exit for all subjects enrolled in FUS II, for testing and assessment (including, but not limited to: pitted red blood cell [pit count], quantitative Howell Jolly Bodies [HJB], glomerular filtration rate [GFR] estimation from the Schwartz equations, cystatin C, urine microalbumin:creatinine ratio [microalbuminuria], VDJ recombination event assessment of genotoxicity, and storage). In addition, all subjects in the "active" arm will be offered re-evaluation at age 10 to include studies with more direct organ function measurement (neuropsychological testing, abdominal ultrasonography, liver-spleen scan, cardiac echocardiogram, transcranial Doppler, pulmonary function testing and GFR estimates) during the five year Follow Up Study II. Only with surrogate and direct measures of toxicity and organ function can we establish appropriate HU treatment paradigms for young children with SCA.

In addition, clinical benefits from long-term HU treatment, such as a decrease in vaso-occlusive pain crises or episodes of acute chest syndrome, may be observed during this follow-up study. The HUSOFT follow-up study (Hankins et al 2005) suggested that HU resulted in a decrease in the frequency of acute chest syndrome (ACS) events, but this analysis was limited by the small number of subjects. Follow-up of a very large group of infants for five additional years, when clinical events are far more common than in early infancy, will allow determination of the role of HU in ameliorating the clinical complications of SCA in young children and provide insight into the risks and potential value of early initiation of such therapy.

Attempts to identify early clinical and laboratory characteristics that predict major complications and organ injury later in life have been only modestly successful. However, markers (early dactylitis, severe anemia, and leukocytosis) identified by the Cooperative Study of Sickle Cell Disease (CSSCD) associated with an increased risk of death, stroke, recurrent pain and acute chest syndrome later in life are parameters likely to be impacted by the early use of hydroxyurea. Based on historical analyses, in an untreated cohort, one might anticipate 20-

30 deaths and 10 to 20 strokes in a cohort of nearly 200 infants followed through the first two decades. Although more recent estimates suggest that nearly 94% of infants with sickle cell anemia will survive to age 18, the potential for morbidity remains high. (Miller et al, 2000; Quinn et al, 2004, 2007 and 2010) At the end of Follow Up Study II we may see a difference in natural history based on treatment with HU. Although long-term outcome may be somewhat obscured by the use of HU at the end of the initial interventional blinded study, any discernible differences on modification of these late major sickle cell-related problems by early, ever or never therapy with hydroxyurea would be of great significance to subsequently born children and their families.

The determination of the overall risk-to-benefit ratio for treatment with HU in this unique group of young children is likely to be complex. Even with the outcomes of the BABY HUG Treatment Study, the safety and efficacy of HU treatment in SCA cannot be fully defined without collection of clinical and at least limited long-term laboratory, organ assessment, and neuropsychological testing follow-up data. Treatment recommendations for primary and secondary prevention based on observational data and expert opinion can be notoriously incorrect (Manson et al, 2003; Wasserthiel-Smoller et al, 2003). A modest treatment effect in the initial BABY HUG Treatment Study may become more apparent and compelling during long-term follow-up. Alternatively, long-term follow-up may demonstrate that the toxicity associated with HU treatment and/or time-limited organ protection (postponement rather than prevention of injury) make its use unwarranted. The initial BABY HUG study and Follow Up Study I cannot fully clarify the role of HU in the treatment of very young children with SCA until the long-term risks and benefits are studied and better understood.

In summary, determination of the long-term risks and benefits of treatment with HU in infants and toddlers with SCA is absolutely essential to perform at this time. The subjects in the BABY HUG Treatment Study represent a unique and “never-to-be-repeated” resource from which to address these issues. The BABY HUG Follow-up Study II will allow more accurate characterization of the role and optimal timing of treatment with HU in young children with SCA.

Follow-up of all subjects for as long as possible is proposed. Initially, all subjects will be followed for at least five years to a common termination point of December 31, 2016 for clinical events, clinically obtained laboratory values, organ assessment tests and neuropsychological assessments.

2.2 SPECIFIC AIMS

The specific aims of Follow-Up Study II are:

1. To identify and define possible long-term toxicities and benefits in children who receive treatment with HU.
2. To determine if prolonged treatment with HU changes the risk and benefits of its use.
3. To investigate the optimal age for initiation of treatment with HU (early vs. late).

2.3 DESIGN OF THE STUDY

After completion of active or passive participation in the BABY HUG Follow Up Study I, each family will be asked to consent to have their child participate in this long-term Follow-Up Study II. If applicable, their child's retrospective data will be collected beginning with the exit visit from FUS I. Follow-up of these subjects will continue to a common termination point of December 31, 2016, and beyond if further funding permits. Depending on the parents/guardians choice of "passive" or "active" follow-up, we will collect some or all of the following parameters that may not be part of routine clinical care: fetal hemoglobin (Hb F); pitted cell counts; Howell Jolly Bodies; BUN, creatinine, bilirubin, LDH, ALT, Brain Natriuretic Peptide (BNP); VDJ and genetic modifiers; stored blood and urine samples; liver-spleen scan, abdominal ultrasound; estimation of the GFR from the Schwartz equations and cystatin C measurements; microalbumin/creatinine ratio; urine osmolality; transcranial Doppler sonography of cerebral blood flow velocity; MRI/MRA of the brain; pulmonary function tests including spirometry; cardiac echocardiography; neuropsychological testing and a questionnaire

regarding subjects' health status related to enuresis, snoring/obstructive sleep apnea and priapism. The plan for the performance of these tests is shown in Appendix A.

All subjects enrolled in the BABY HUG Follow-Up I Study who participated for at least 24 months are eligible for the Follow-Up Study II regardless of their initial treatment assignment, their current treatment status, their prescribed dose of HU and perceived compliance, or the Follow-Up Study I group (active versus passive) selected. Subjects who selected the "passive" group in FUS I are permitted to select the "active" group in FUS II and vice versa. Subjects receiving stem cell transplantation will be excluded from participation in the Follow-Up Study II but will be assessed annually for vital status and graft-versus-host disease. Subjects receiving other therapies for their SCA, including chronic transfusion, are eligible for participation in BHFU II, although their data may not be appropriate for inclusion in all analyses. For example, it would be more appropriate to analyze those on chronic transfusion as a separate subgroup for number of clinical events such as pain crises or acute chest syndromes for the time intervals they were on chronic transfusion. Although the BABY HUG Follow-Up Study II is intended to monitor the longer-term risks of HU therapy, it will also test the hypothesis of the impact on safety and efficacy of early versus late initiation of HU. Experience from the MSH study (Steinberg et al. 2003) suggests that important covariates to be studied in the BABY HUG Follow-up Study II include the unrandomized comparisons of ***ever*** used HU versus ***never*** used HU, and a time-dependent covariate indicating whether a subject is "on" or "off" HU treatment during follow-up. The assessment of this last variable will require careful collection of HU treatment information over the course of the Follow-Up Study II and enumeration of significant clinical adverse events. The data will be collected by the dedicated study personnel at each Clinical Center, using semi-annual review of the subject's medical record. Data to be abstracted and recorded on structured reporting forms includes (but is not limited to) results of physical examinations, interim history assessments, hospital/Emergency department visits and local laboratory assessments. It is likely that subjects in the BABY HUG Follow-Up Study II will continue to be

medically followed at their Clinical Center by physicians and nurses knowledgeable about and dedicated to the goals of the treatment study, making the probability of long-term local data submission feasible and extremely probable. Since continued HU treatment will not be randomized, it will not be possible to itemize all of the treatment and response trajectories that may occur in this study and statistically assess each possibility. Instead, the Follow-up Study II statistical design will have a generalized and comprehensive analytical plan that will allow investigators to detect changes in these trajectories and associations as Follow-Up Study II continues.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY II PROTOCOL

CHAPTER 3

SUBJECT ELIGIBILITY, RECRUITMENT, ORIENTATION, AND INFORMED CONSENT

3.1 INTRODUCTION

All subjects who were enrolled in the Follow-up Study I who participated for at least 24 months will be eligible for Follow-Up Study II except if they received a stem cell transplant. A new informed consent from parents or guardians will be required for participation. When families consented to enrollment in the BABY HUG Treatment Study, parents/guardians were made aware that the investigators wished to maintain contact with the child after the treatment study ended, and because most subjects were clinically followed in the same Clinical Center where they participated in the BABY HUG Treatment Study, this was thought to be realistic. Information about the concept of a follow-up study was repeated during the course of the study and subject's parents/guardians consented to Follow-Up Study I. During the Follow-Up Study II, passive data collection with retrospective data abstraction (including information from other health providers when necessary) will be carried out for all subjects whose parents/guardians give consent to be in the Follow-up Study II and who provide assent (if age-appropriate). In addition, blood cells, serum, and urine will be collected for measurement of important surrogate markers of efficacy and toxicity at Follow-Up Study II entry and exit. Some of the optional "active" assessment studies (as described in the consent form) are more than minimal risk, but will allow ongoing evaluation of organ function both for absence of new toxicities as well as potential for preservation of function that would usually be lost by children with sickle cell anemia in early childhood.

3.2 FAMILY ORIENTATION

At the end of the BABY HUG Treatment Study all subjects' parents/guardians were offered treatment for their child with open-label HU by their child's physician. Most

parents/guardians made this decision without knowing their child's randomized BABY HUG treatment study assignment.

The rationale and the importance of the Follow-Up Study II will be presented and explained to the parents/guardians. They will be given a list of the passive follow-up data to be collected and the active assessment evaluations and tests that would be performed (see Appendix A). They will be advised that they are required to sign a new consent form in order to participate in the Follow-up Study II. If age appropriate, the subject's assent will also be obtained. Open-label HU treatment will be managed according to local clinical care standards. Information about HU treatment will be collected on Follow-up Study II forms. Parents/guardians will be advised that the Follow-up Study II will not be paying for open-label HU treatment.

Since participation ended in the randomized trial, subjects may have been on and off HU, chronic transfusion, or other therapies for extended periods. Families are free to select, in consultation with their child's hematologist, any SCA treatments and still be followed by BHFU II. Study participation will be categorized by the treatment they are receiving at the end of each data abstraction interval. Patients receiving a stem cell transplant will no longer undergo regular study visits or procedures after they begin the conditioning regimen. They will only be assessed on an annual basis for vital status and graft versus host disease. Families may continue to participate in the study even if they move out of the area of the Clinical Center. They will be encouraged to notify the coordinator in advance of the move so that plans for passive data collection in their new location can be developed.

3.3 INFORMED CONSENT

Individual Clinical Center consent and assent forms will be prepared based on the model forms presented below. The model consent and assent forms will be approved by the Observational Safety Monitoring Board (OSMB) prior to their release to the Clinical Centers for submission to the local IRBs. Each final consent and assent form will be reviewed by Data

Coordinating Center (DCC) staff and NHLBI Program staff to ensure all required elements of the forms have been addressed.

The Clinical Center Principal Investigator (PI) will attempt to obtain consent from each family in the BABY HUG Follow-Up Study I by contacting the parent/guardians following approval of FUS II by the Clinical Center Institutional Review Board, the DCC, the NHLBI and the OSMB. The family will be given adequate time and privacy to review the consent form. They will have the opportunity to have all of their questions and concerns addressed by the PI. An ombudsman, required for the BABY HUG Treatment Study consent process, may be present but is not required. A copy of the signed, dated and timed consent form will be given to the parent/guardians and placed in the child's medical record. The original will be maintained in study files by the Principal Investigator or designee at each Clinical Center.

3.4 INFORMED CONSENT TEMPLATE

****Those items appearing in BOLD or ITALIC typeface are those items which are required by the NHLBI to be included in each Clinical Centers' specific consent form. ****

PURPOSE, PROCEDURES AND LENGTH OF STUDY

We are asking you to agree to your child's participation in the BABY HUG Follow-Up Study II. This study in up to 14 Clinical Centers will follow the 163 subjects who were enrolled in the BABY HUG Treatment Study and Follow-Up Study I to learn about the long-term effects of study treatment. This study is sponsored by the National Institutes of Health: National Heart, Lung, and Blood Institute (NIH-NHLBI). If you agree to allow your child to **enroll in the BABY HUG Follow-up Study II, we will collect medical information on your child until at least December 31, 2016.**

The BABY HUG Treatment Study was designed to see if treatment with the drug hydroxyurea (also called HU) in children with sickle cell disease could prevent organ damage, especially in the spleen and kidneys. There was also a chance that treatment could prevent painful crises, lung disease, stroke, and blood infection. Since your child exited the BABY HUG Treatment Study, you and your child's doctor have decided whether or not to give your child HU from the pharmacy. In Follow-Up Study I, your child was followed carefully with medical and laboratory testing to begin to learn about the longer term benefits and risks of HU treatment. *The main goal of the Follow-Up Study II is to study the longer-term safety and efficacy of HU treatment in children.* If treatment with HU provides long-term benefit or if a safety problem is discovered, it will be important to determine if the age that HU was started is important. Follow-up of your child will provide us additional important information about whether HU treatment should be given to infants and if so, the best age to begin treatment. **We would like your child to join Follow-Up Study II whether or not you choose for him or her to take HU from the pharmacy now or ever.**

You may choose to let your child participate in the BABY HUG Follow-up Study II in one of two ways. **The first is "passive" follow-up.** As your child is seen for usual clinical care of sickle cell disease, we will **collect information from routine tests ordered by your child's clinical sickle cell doctors**. These tests usually include a complete blood count and measurement of your child's height and weight at each visit. At least twice a year we will also collect information about illnesses that your child has had since the last visit and how they were medically treated. We will also record the results of TCD (Transcranial Doppler or brain ultrasound) tests, imaging by CT or MRI, other medical tests and any clinical consultations if ordered by your child's doctor. We will also ask you questions regarding your child's health status related to enuresis (bed-wetting), snoring/obstructive sleep apnea and priapism (unwanted erections of the penis in males).

We will collect **four teaspoons of blood and a urine sample from your child at the beginning and end of this follow-up study**. These samples will be used to assess spleen and kidney function as well as potential changes in your child's genetic material or DNA. **The blood samples will not have your child's name on them and will be stored for use as new laboratory evaluations become available for future research on sickle cell disease and related disorders. We would like to store these unnamed blood samples <u>forever</u>.**

The second way you may choose to have your child participate is known as "**active" follow-up.** The same blood and urine samples will be collected, and we will ask you the same questions regarding your child's health status as outlined in the above paragraph. Your child's health information will be collected and submitted to the BABY HUG coordinating center at least twice per year as with passive follow-up. **In addition, you agree to allow, at one time, when your child turns 10 years old, imaging studies, and neuropsychological and behavioral testing, some of which are similar to those done at the beginning and end of the BABY HUG Treatment and Follow-Up I Studies.**

If you choose to participate in either group, we will collect information from your child's medical record since he/she exited the BABY HUG Follow-Up I Study. Whether you choose the active or passive group, all of your child's stored blood samples will be de-identified but linked to the treatment study and Follow-Up I study datasets and will be available to researchers for future studies of sickle cell and related diseases.

At the end of the Follow-Up Study II, your family will be informed of the results and any new recommendations from the study doctors.

STUDY TESTS TO BE PERFORMED

Passive Follow-Up Group

We will collect information from your child's medical record including height and weight and the results of physical examinations and laboratory tests performed as part of routine clinical care. If your child has had any major health problems, imaging studies, or medical consultations between clinic visits, including the usual complications of sickle cell disease and especially those that require hospitalization, we will collect that information. If TCD testing has been ordered clinically by your child's doctor, we will collect all TCD results and centrally review the images of one TCD test during the study. In the Follow-up Study II, all data collection will end on December 31, 2016**.**

The information collected from testing your child's blood samples will be added to the BABY HUG Treatment Study and the Follow-up Study I data files for analysis. The laboratory tests performed as part of the Follow-Up Study II are not diagnostic tests for clinical disease. The results will be used as part of a large group analysis without identifying your child. **You will not receive the research results from your child's individual sample.** Knowledge of the test results will not change your child's current medical care.

If your family moves from this area, the study coordinator will contact you by telephone or in writing about every 6 months to obtain information about your child's growth and development and clinical events. We would like to obtain any information relevant to the Follow-

up Study II regarding your child's health from other health care providers. We will ask you to sign a release for us to obtain that information.

Active Follow-up Group

If you agree to have your child in the active follow-up group, the same clinical information, blood and urine specimens, and questionnaires related to your child's health status as in the passive follow-up group will be collected, but there will be additional blood and urine tests (some tests will be performed at specialty research labs throughout the course of the study), as well as tests to check the function of your child's brain, spleen, kidneys, brain, heart, and lungs. These additional tests will be performed only once, at the time your child turns 10 years old. Each of these tests may require a separate visit and include:

- Liver Spleen Scan
- Abdominal Ultrasound
- Brain MRI/MRA
- Cardiac Echocardiogram/Pulmonary Function Testing
- Transcranial Doppler
- Neuropsychology Testing (Vineland, WISC-IV, Connor CPT II and Peds QOL)

A test requiring a small dose of radiation to evaluate the function of the spleen will be performed in exactly the same way it was done in the BABY HUG Treatment Study (liver/spleen scan). For this test, small doses of radioactive material will be given through your child's vein. We will use a camera sensitive to radioactivity to take pictures of your child's spleen. The radioactive material will leave your child's body in urine or stool by the next day. In order to monitor your child's brain growth and development, neuropsychological testing including WISC-IV, Connor CPT II, and Peds QOL will be performed. There will also be a behavioral test, called the Vineland Adaptive Behavior Scale (also done previously in the BABY HUG Treatment and Follow-up Study I), performed when your child is 10 years old. Your child will also have an ultrasound (sound wave) imaging test of spleen and liver size and a Transcranial Doppler Study

of the brain. These are the same tests that were performed twice in the BABY HUG Treatment Study and possibly once in the Follow-up Study I. There are no risks associated with the use of sound waves used for ultrasound of the liver, spleen or brain. Blood tests for potential damage to your child's DNA will be performed at the beginning and the end of the Follow-Up Study II at a specialized laboratory.

We will take magnetic resonance image (MRI) and magnetic resonance angiography (MRA) pictures of your child's brain to check its growth and to look for strokes that can occur in some children with sickle cell disease. The MRI/MRA doctors will talk to you to explain the pictures every time they take them. Your child will be lying down on a small bed. The bed moves into a small tunnel about 6 feet long and 2 feet across. Your child will hear some loud machine noise. Ear plugs can be used. During this test, someone in the room can talk with your child taking the test or touch your child, if your child wishes it. Once in a while, children cannot finish this test because they get scared or afraid of small spaces, but the test is not dangerous or painful.

We will also perform a test called a cardiac echocardiogram. The echocardiogram takes moving pictures of your child's heart using sound waves, much the way that the ultrasound does. Unlike x-rays, the echocardiogram does not use radiation; instead it uses sound waves. A computer analyzes the results and takes pictures of the inside of the heart. By taking these pictures, the doctor can make measurements on your child's heart. These measurements help the doctor to describe how well your child's heart functions. For the test, your child will be placed on a table and a doctor will put a clear jelly on your child's chest. A sensor (a machine that looks like a microphone) will be placed on your child's chest and it will be moved around. A picture of your child's heart will appear on a television screen and a tape recording will be made of this picture. You may also hear a whooshing sound coming from the television. This is the sound of your child's blood flowing through your child's blood vessels.

We will be performing Pulmonary Function Testing which involves testing of your child's lungs to measure how much air he/she breathes in and out. These tests involve your child breathing into some machines so that we can see if he/she has any signs of lung problems. Your child will also be given a breathing treatment using a medicine (albuterol or Xopenex®) that he/she breathes in from a small machine in order to see if his/her breathing improves. If there is improvement, this could be a sign of a lung problem. No experimental medicines will be used during this testing. This testing will take about 1 ½ hours.

Some of the "active" tests will be performed once every year your child remains in the study. Those tests include an extra blood sample to assess spleen and kidney function and a physical examination to assess your child's growth.

RISKS

The needle used to take blood may cause a sharp pain as it goes into the skin. Sometimes a bruise will form at the place the needle goes into the skin.

If your child is enrolled in the active follow-up group, he or she will have some medical imaging studies. The liver-spleen scan test requires that an IV be started in your child's vein and that a small amount of radiation (like the amounts that people encounter naturally in daily life from space) is injected into your child's body. Like the blood draw, the risks of the needle stick for the IV are pain and bruising. Your child will receive about the same amount of radiation as he or she would get from living in our natural surroundings for about nine days. The radiation dose is what your child will receive from the Follow-up Study II only and does not include any exposure he or she may have received or will receive from other tests. The magnets and radio waves used in the MRI/MRA have not been seen to cause any side effects. There may be harmful effects that we do not know now but may learn about in the future. There are no known risks to your child as a result of having an echocardiogram performed.

BENEFITS

At this time no one knows whether HU will help your child over many years. The results of Follow-Up Study II, along with those from the BABY HUG Treatment Study, will help doctors decide in the future if and when to give HU to young children with sickle-cell disease and how long to give it.

FREE CHOICE

Your child's participation in the Follow-up Study II is up to you. You are free to take your child out of this study at any time. You are free to start or stop HU from the pharmacy, after talking with your child's doctor at any time. You are free to choose to have your child participate in the active or passive group, to have your child's information included in the data file, to decide if your child's blood sample can be saved indefinitely and used for future research, and to decide if DNA testing is performed on your child's blood samples**.** If you take your child out of the study or do not take part in the study, we would still like to provide medical care for your child. That is, you may choose not to be part of the Follow-up Study II, not allow us to collect information from that care, and just allow your child to receive standard care for sickle cell disease in this hospital and clinic. You and your child's doctor may plan to use or not use hydroxyurea. Other treatments may be possible for your child. These treatments include chronic blood transfusions and stem cell or bone marrow transplantation. Your choice to continue in the Follow-up Study II will not change the way your child is treated in this hospital or the treatments available to your child. In or outside of the study, we want to give the best care for your child.

COSTS

All costs that are considered part of routine clinical care will not be paid by the study. Routine care will be billed, as before, to you and/or your health insurance provider. **Costs related to the blood and urine samples obtained just for this study, and procedures performed in the active follow-up group, will be paid for by the study. If you decide to**

have your child take hydroxyurea, the costs of the medication and the laboratory studies to monitor its effects will be billed to you and/or your health insurance provider.

PAYMENT

If you choose to allow your child to participate in the active follow-up group you will receive $(_____: To be completed by clinical site staff) for the extra clinic visits required. Payment will be made when you visit the clinic for the start of the active group tests. This is to cover your costs of parking, travel, meals and other expenses to get your child to the clinic for these extra tests. Payment will not be given for your child's regular clinic visits.

PRIVACY

Your child has a right to privacy. All information in this study that can single out your child or your family will remain private. A numbering system that does not allow anyone outside this Clinical Center to know your child's name will be used to identify your child. Your child will not be named in reports of results from this study. Your child's medical reports and family data will remain private

We will do everything we can to keep your child's medical and research data private. Specifically we will do the following things to maintain your privacy:

- *Though efforts will be made to remove identifying information from images that leave our institution, there is a chance that identifying information will not be able to be removed from the copies of the images of the liver spleen scan, abdominal ultrasound, brain MRI/MRA, cardiac ECHO and/or Transcranial Doppler ultrasound when these files are sent to the places where study physicians will interpret the images for the study. Study records that identify your child will be kept private as required by law.*
- *There are laws (Federal Privacy Regulations) to protect your privacy. Your child will not be identified by name, social security number, address, telephone number, or any other*

*direct personal identifiers in study records sent outside of **this institution** except when required by law or as described in this consent.*

- *Your child has been given a study identification (ID) number. All study records and questionnaires will be labeled with this number and not your child's name or other personal data when they are sent outside of this institution. The files that link the ID number to your child will be kept in a locked, secure area in this center that only the study team can open.*
- *A Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you or your child based on his or her genetic information. This law generally will protect your child in the following ways:*
    - *Health insurance companies and group health plans may NOT request the genetic information that we get from this research.*
    - *Health insurance companies and group health plans may NOT use the genetic information that we get from this research when making decisions regarding eligibility or premiums.*
    - *Employers with 15 or more employees may NOT use the genetic information that we get from this research when making a decision to hire, promote, or fire your child or when setting the terms of your child's employment.*
    - *This Federal law does not protect your child against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.*
- *Data gathered during this study and medical records may be checked and verified by staff of the NHLBI, <**site/institution**>Institutional Review Board, or New England*

Research Institutes (NERI), the BABY HUG Data Coordinating Center. All medical records from this site or from other institutions that have personal identifiers will be treated as private and will be shared only with these agencies, or as required by law.

- *The results of this study may be published for all the subjects as a group but will not identify your child individually.*

A description of this clinical trial will be available on www.ClinicalTrials.gov as required by U.S. law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

CONSENT FOR A DATA FILE

At the end of the Follow-up Study II, a computer file of the study results will be made for future use by researchers studying sickle cell and related diseases. The information collected from your child's participation in the Follow-up Study II, all testing done during the study, and the saved blood samples will be linked to the child's participant number in the Follow-up Study II data file. This data file will not have your child's name, your name or any facts that could be linked to your child or family directly. The computer file may be used by other doctors to study sickle cell disease or related disorders. Data may be given to the National Institutes of Health, the Food and Drug Administration (FDA) or other U.S. or state agency as required.

I ☐ agree ☐ do not agree _____ for the data file to include my child's information.
Initials

CONSENT FOR STORED BLOOD AND URINE SAMPLES

These are the blood and urine samples taken at study entry and exit.

I ☐ agree ☐ do not agree _____ to the saving of my child's blood and urine

Initials **samples indefinitely.**

I ☐ agree ☐ do not agree _____ Initials to the use of my child's blood and/or urine samples for future research on sickle cell disease and related disorders.

I ☐ agree ☐ do not agree _____ Initials to DNA testing of my child's blood samples.

CONSENT

I agree for my child to participate in the ☐ _____passive follow-up group.
Initials

OR I agree for my child to participate in the ☐ _____ active follow-up group.
Initials

LIMITATIONS

The <<insert Clinical Center name>> will not provide compensation for subjects who may incur injuries as a result of being in this research except as is required by law. This means that while all study doctors will do everything possible to provide careful medical care and safeguards in the conduct of this research, the medical center will not offer to pay for injury resulting solely from the research itself. The study sponsor, The National Heart, Lung, and Blood Institute, does not offer financial compensation or payment if you are injured as a result of participating in this research study.

You can discuss the rights of children who participate in research with the Chairman of the Medical Center's Institutional Review Board, telephone number ( ). This board is composed of doctors and lay people who have reviewed and approved this study. Dr. _____, Principal Investigator of this study, is also willing to talk about any of your concerns about the study at telephone number ( ).

What will happen if your child is injured by this research?

All research involves a chance that you might get sick or suffer an injury directly related to your participation. If such problems occur, please contact the researcher listed above. They will help you get the necessary medical care. This care does not imply any negligence on the

part of this hospital or the researchers. By signing this form, you do not give up any of your legal rights.

COPY OF CONSENT

If you agree to have your child take part in this research study, you will receive a signed copy of this consent form.

PARENT, INVESTIGATOR AND WITNESS SIGNATURES

I have read all of the consent form. I have been given a chance to ask questions and have received answers about areas I did not understand. I willingly give my consent for my child to join this study.

I understand that I may withdraw my child from the study at any time. In doing this, I will, in no way, change my child's ongoing medical care at this medical center or elsewhere.

_____	_____	_____	_____
Child's name	Signature of parent or legal guardian	(Date)	(Time)
	_____	_____	_____
	Signature of parent or legal guardian	(Date)	(Time)
_____	_____	_____	_____
Investigator's name	Signature of Investigator	(Date)	(Time)
_____	_____	_____	_____
Witness' name	Signature of Witness	(Date)	(Time)

<Institution>

BABY HUG FOLLOW-UP STUDY II

Assent (7 – 11 year old children)

We want you to be in a research study so we can learn more about your sickle cell disease. Your doctor, told you that sickle cell disease is a blood problem that you have had your whole life.

As part of our study, we will ask you questions about how you are feeling and ask your doctor or nurse about how you are doing. You will be asked to have a blood test done, and other types of tests may also be done. We would like your OK to perform these tests.

We will take blood from your arm and collect a urine sample. The poke for the blood may hurt a little for a short time. Some of the blood will be used to grow cells, and the blood cells and urine sample will be studied for many years to learn more about sickle cell disease. We are asking for your OK to keep your blood cells and urine sample in the laboratory. If you say yes, you might help doctors find new ways to help other children with sickle cell disease. If you say no, that is OK too, we will not grow your blood cells or study your urine sample.

Talk with your parents or guardian before you make up your mind about being in this study. We will also ask your parents or guardian to give their permission for you to be in the study. Even if your parents or guardian say "yes", you can say "no" and we will not put you in the study.

Being in this study is up to you. No one will be mad if you don't want to be in it or even if you change your mind later and want to stop.

You can ask any questions that you have about the study. If you have a question later on that you didn't think of now, you can call Dr. _____ at _____.

Putting your name on the line means that you agree to be in this study. We will give you and your parents or guardian a copy of this form after you have signed it.

_____ _____

Name of Subject Date

_____ _____ _____

Signature (for written assent) Age Grade in School

_____ _____

Signature (for verbal assent) Date

_____

Name of Investigator Obtaining Consent

_____ _____

Signature of Investigator Obtaining Consent Date

PEDIATRIC PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY II PROTOCOL

CHAPTER 4 STUDY ENDPOINTS

4.1 INTRODUCTION

The primary objective of the BABY HUG Randomized Trial was to determine the safety and effectiveness of HU in the prevention of chronic end organ injury in young children with sickle cell anemia (SCA). The primary selected organs and indices of damage were: worsening splenic function as defined by decline in radionuclide uptake and continuing renal damage defined as an increase in GFR measured by DTPA. Although the initial study did not demonstrate this level of benefit for the indices of damage in these two organs, it did clearly demonstrate that HU significantly reduces the number of clinical events in children with SCA. Children who received HU did show less spleen failure than those treated with placebo, however the results were not statistically significant.

With a clear finding for efficacy in clinical events, it is important to continue follow up of the BABY HUG subjects to determine if the clinical benefit is maintained over time while surveying for known and not-yet-apparent toxicities. However, determination of the effect of HU on organ failure remains an important aim of FUS II.

The primary objective of Follow-Up Study II is to monitor the continued safety and potential efficacy of HU treatment. Safety of HU will be assessed by ongoing clinical monitoring of growth and development, age-appropriate neuropsychological evaluation, serial hematologic and chemistry parameters, and the frequency of expected and unexpected clinical events related to sickle cell anemia . For this reason follow-up should continue as long as practically possible. Disease and treatment-related effects on the spleen will be measured by a liver-spleen scan, pitted cell counts and HJB enumeration. Kidney function will be measured by GFR estimation from the Schwartz formula or cystatin C measurements and the urine osmolality and

urine microalbumin:creatinine ratio. The BH FU II investigators will also seek to study how early treatment with HU changes the child's disease trajectory on open-label HU.

4.2 OTHER FOLLOW-UP ENDPOINTS

4.2.1 Growth, Development, and Education

Height, weight, physical examination parameters (for example the presence of a palpable spleen or liver) are routinely assessed at clinical care visits which usually occur every 3-4 months through the first five years of life and every 6-12 months at older ages in children with sickle cell anemia . Physical examinations will be conducted specifically for Follow-Up Study II at entry, exit, once per year and as other clinically-directed exams are performed in the "active" group. Clinically-directed physical examination data will be collected in the "passive" group. Subjects will be carefully monitored for height or weight percentile and compared to standardized growth curves. In particular, the growth curves of subjects ever exposed will be further divided into those who have taken HU both in the treatment study and Follow-Up Study I and II versus those treated with HU only in Follow-Up Study I and II and compared to those never on HU. (HU subjects in the treatment study whose parents elect not to have their child take HU in the Follow-up Study II will be excluded from this analysis.)

Careful evaluation of the BABY HUG subjects through the first decade of life for growth, school performance, development of pubertal characteristics (menarche and Tanner Score), and fertility is highly desirable. During the period demarcated for Follow-Up Study II, the first subjects may complete the eighth grade, although the majority will complete the fifth grade. We will collect self-reported data about school placement (special education placement and/or repeated grades vs. those continuing without such assistance) and the presence or absence of parental concern about language development and behavior.

4.2.2 Hematologic and Clinical Events

Complete blood counts (CBC) will be collected for all subjects at their regular clinic visits

in accordance with standard clinical practice. Creatinine and hepatic transaminase values are clinically obtained for children on hydroxyurea. The investigators will continue to compare subjects on and off of treatment with HU to assess the effect of HU treatment on the absolute neutrophil count, white blood cell count, mean cell volume, reticulocyte count and platelet count.

For any subjects having serious clinical events, these events will be recorded including, but not limited to, stroke, acute chest syndrome, splenic sequestration events (and presence of a palpable spleen), transfusions, and hospitalizations. This information will be collected by structured, retrospective review of the medical record at the semi- and annual reporting periods.

4.2.3 Fetal Hemoglobin

Maintenance of an elevated fetal hemoglobin with hydroxyurea therapy is one of the secondary endpoints of the BABY HUG Treatment Study. Fetal hemoglobin will be measured as part of clinical care of study subjects in Follow-Up Study II. Measurements will be performed yearly in the active group; at study entry and at the end of the study in both the active and passive groups and more frequently as dictated by clinical practice. The levels of fetal hemoglobin observed will be compared to the serial values available from the BABY HUG Treatment Study and Follow-Up Study I data to evaluate the persistence of a beneficial effect from hydroxyurea.

4.2.4 Central Nervous System

Neuropsychological evaluation with the Wechsler Intelligence Scale for Children (WISC-IV), the Connor CPT II, the Peds QOL (PedsQL 4.0 generic core, SF 15), a sickle cell disease specific QOL test, and the Vineland Adaptive Behavior Scale tests will be performed once each in Follow-Up Study II in the active group. Results will be analyzed with comparison to the previously collected Bayley and Vineland tests in the BABY HUG Treatment Study to carry out an analysis of covariance. Longitudinal profiles will not be analyzed because the

neuropsychological scales must be changed as the child ages, which will prevent the creation of a neuropsychological trajectory based on a common measurement.

The anatomic integrity of the central nervous system will be evaluated by magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) in the active group once during the Follow-up Study II. Brain volume (global), lobar and cerebellar volumes, cerebral infarctions (location, count and volume), stenoses/occlusions of major cerebral arteries and gliosis or scars will be compared to those MRI/MRA scans which were performed in the BABY HUG Treatment Safety and Feasibility Study and between treatment groups (HU vs Placebo in BH and HU vs other clinical care in BHFU I).

Transcranial Doppler (TCD) studies are performed as part of routine clinical care in sickle cell anemia patients since TCD velocity is considered a surrogate marker for stroke risk in this population. One TCD study which is performed during the Follow-up Study II (the one performed closest to 10 years of age) will be interpreted by the Central TCD Laboratory at Medical University of South Carolina and the results will be compared to those TCD studies performed during the BABY HUG Treatment Study and Follow-up Study I.

4.2.5 Spleen

The liver-spleen scan will be performed on subjects in the active follow-up group at 10 years of age. The results of this scan will be compared to the scans performed at initial screening and exit from the BABY HUG Treatment Study and during the Follow-Up Study I. The follow-up scan will be categorized by the same panel of nuclear medicine specialists who read the previous scans. Once again these reviewers will be masked to all treatments received by the subject and will not be involved in the acquisition of the images.

Subjects will be compared between groups for the continued presence of a spleen with sufficient function to take up the radionuclide. A functional spleen can be seen as both having potential for toxicity (the child remains at risk for splenic sequestration, a potentially fatal

complication that children with sickle cell anemia usually do not develop after 3-5 years of age) as well as demonstration of HU's ability to preserve organ function.

Pit counts and HJB quantitation will be performed at entry and exit for all subjects, during the Follow-Up Study II. Subjects who agree to active follow-up will have additional pit count and HJB measurements once per year. The results of the pit counts will be compared with those serially performed during the BABY HUG Treatment and Follow-Up I Studies. This will provide an assessment of changes that have occurred over the entire period of study. The results for the HJB will be compared with those performed at screening and completion of the BABY HUG Treatment and Follow-Up I Studies. This will provide an assessment of serial changes in surrogate measures of spleen function. The non-invasive markers of spleen function will be compared to the results of the liver-spleen scans (when available) to continue to assess their reliability in providing the same information about ongoing risk from a functional spleen as the radionuclide scan.

In addition to the centralized qualitative scoring of spleen scans as present, decreased and absent, quantitative measures (quantitative nucleotide uptake, HJB counts and pitted cells) will again be performed in FUS II. These measures have been shown to correlate well with the qualitative assessment, and may offer more precise estimates of the continuous decline in spleen function that occurs in young children with sickle cell anemia .

BABY HUG Follow Up II Investigators will develop a summary measure utilizing all of the quantitative assessments that are scheduled to be repeated in BABY HUG FU II. Multivariate analyses will determine the optimum combination of results to quantitate splenic function over time.

The development of this measure will begin by performing a factor analysis on these assessments collected in the BABY HUG FU II Study. This analysis will be used to reduce these variables to two or one summary measures representing the best explanation of the overall multivariate variation in the measures. The measurements will be compared to the

qualitative evaluations of splenic function to assess external validity of the resulting measures. Once validated, these measure(s) will be used to track the trajectory of splenic function over time from the first evaluation at entry to the BABY HUG Randomized Trial to the completion of BABY HUG Follow up II using longitudinal data analysis.

Subsequently, analyses will demonstrate which of the markers (quantitative uptake, pit counts or HJ bodies) best independently assess splenic function in future studies.

4.2.6 Kidney

Measurement of serum BUN and creatinine will be performed in order to estimate the GFR according to the Schwartz formula, GFR=kL/Scr, where L = the body length in centimeters, Scr = the serum creatinine concentration in mg/dL, and k = 0.41 mg creatinine/100 min x cm x 1.73 m^2. Blood Urea Nitrogen (BUN) and cystatin C will also be collected to calculate a recently reported Schwartz GFR. The newly developed Schwartz formula is: GFR(mL/min per 1.73 m(2))=39.1[height (m)/Scr (mg/dL)](0.516) x [1.8/cystatin C (mg/L)](0.294)[30/BUN (mg/dL)](0.169)[1.099](male)[height (m)/1.4](0.188).[1]

Measurement of serum cystatin C will also be used to estimate the GFR. Cystatin C measurement was added to the BABY HUG Treatment Study in October 2006 due to its apparent superiority over creatinine-based estimates of GFR (Alvarez et al, 2006). This may be especially true in individuals with sickle cell disease due to their substantial tubular secretion of creatinine. This measurement was obtained, to the extent possible, on residual samples of serum stored from BUN and creatinine measurements in the treatment and FU I studies. Measurement of cystatin C (by blood sample) will be performed by the Clinical Chemistry Laboratory at Cincinnati Children's Hospital Medical Center and will be performed at entry into the Follow-Up Study II and at the end of the study. Subjects in the active group will have an additional determination once per year during the Follow-Up Study II. As discussed in the BABY HUG Treatment Study protocol, the development of hyperfiltration is one of the earliest

lesions in sickle cell nephropathy. Evaluation of renal function, particularly raw creatinine and/or cystatin C values, will also allow assessment of any unexpected detrimental effects of HU therapy.

Microalbuminuria, assessed by the urinary microalbumin to creatinine ratio, may also be regarded as an early sign of sickle nephropathy (Darnidharka 1998). This ratio will be measured in all subjects at entry to this study and at the end of this study. Assessment of this parameter will provide additional information about the toxic or beneficial effects of HU on kidney function in young children with sickle cell disease. For example, even if HU therapy prevents the rise in GFR or hyperfiltration, it may make other aspects of renal function, such as microalbuminuria, worse. Again this assessment will allow determination of the true spectrum of toxicity or benefit conveyed with early use of HU.

History in the form of a questionnaire will be taken every 12 months to assess for enuresis in both the active and passive groups. Urine osmolality will be measured after overnight deprivation at study entry and exit in the active group.

4.2.7 Cardiac Evaluations

Echocardiograms performed as part of the BABY HUG follow up study are**: for research purposes only,** will not include a clinical interpretation from the central echocardiogram reading center and the CD-Rom with the echocardiographic data will not be returned to the clinical center. The echo measurement data generated at University of Miami will be returned to the site. However, there may be a delay in doing the measurements and reporting them to the site. Therefore, if there is a clinical indication for an echocardiogram, the site may wish to perform a complete clinical echo locally. If a clinically-indicated echocardiogram occurs within a study window, the echocardiogram can be used for the study as long as the original data are available for central reading and all of the components of the echocardiogram that are required are included.

Measurement of height and weight are critical to the interpretation of the echocardiographic measurements. Height and weight should be measured on the day of the echocardiogram.

The echocardiogram requires a high-resolution ultrasound machine using a transducer appropriate for body size and equipped with pulsed Doppler, 2-D directed M-mode, tissue Doppler, and ECG. Recording of systolic, diastolic, and mean blood pressure requires an automated blood pressure recorder such as the Dinamap Vital Signs Monitor, Critikon, Inc.

To assure full compliance with HIPAA requirements, no patient identifiers (PID) are to be included in the submitted echocardiograms. The PID and the date of the echocardiogram will uniquely identify each of the exams. These data should be embedded in the image data in addition to inclusion on the echocardiogram submission form to assure participant identification. The method of inclusion of the PID depends on the specific ultrasound machine but entering the PID as the patient last name will generally assure that the information will be embedded on the image.

Methods. The 2-D echo and Doppler exams are preferentially recorded in DICOM format and transferred to a CD or DVD for submission to the core lab. Alternatively, for those clinical sites without digital recording capability, the study can be recorded in super-VHS video format on ½" videocassette tape with submission of the original recording for analysis. Original videotape recordings submitted to the core lab will be converted to digital format at the core lab and the videotape will be returned to the clinical site.

All measurements will be made at the core lab and no measurements are required from the clinical sites. A good quality electrocardiographic recording is required to be present on all echocardiographic images. Each of the individual recordings should include 6-8 cardiac cycles in order to include at least 2 respiratory cycles. M-mode and pulsed Doppler recordings are used to measure time intervals and therefore should be recorded at a sweep speed of 100mm/s. The following recordings should be obtained:

1. *Height and weight should be measured on the day of the echocardiogram.*
2. *Apical long-axis 4-chamber view of the left ventricle.*
3. *Two-D parasternal long-axis images of the aortic root.*
4. *Two-D parasternal long-axis images of the right ventricle inflow tract (RVIT).*
5. *Two-D images of the left ventricular short axis*.
6. *Two-D directed m-mode.*
7. *Pulsed spectral Doppler samples of the mitral valve inflow.*
8. *Pulsed spectral Doppler samples of the tricuspid valve inflow.*
9. *Apical 4 chamber view of the right ventricle*.
10. *Tissue Doppler.*
11. *Aortic Doppler.*
12. *Pulsed spectral Doppler samples of pulmonary vein inflow.*
13. *M-mode color-Doppler recording of the left ventricular diastolic flow propagation velocity.*

Blood Pressure Measurement. Blood pressure (BP) should be obtained simultaneously with the m-mode recording since it will be used for wall stress calculation. When an automated blood pressure device is used, the device can be switched on and allowed to run during the m-mode sample, with a total of 4 blood pressure recordings. The first recording is the least accurate as the machine uses this value to set the target range for future samples, and is therefore discarded. The subsequent 3 samples of systolic, diastolic, and mean blood pressures are to be submitted.

Interpretation. Videotape or digital recordings and blood pressure data will be sent to a central reading center (Children's Hospital Boston) for analysis of LV size, function, loading conditions and contractility.

No local interpretation is required**,** nor is a clinical interpretation returned to the site. The following list provides the number and types of images that will be required by the central reading center staff in order to fully read the data for the Baby Hug required outcomes.

Baby HUG FUS II: Estimate and breakdown of how many echoes should be included (minimum of about 29 total images) for a complete evaluation

1. ***2-D Parasternal long axis view of the Aortic Root*: At least 1 image**

- Parasternal long axis without zoom
- Parasternal long axis with zoom

2. ***2-d Parasternal Long-axis of the RVIT (tricuspid valve)*: At least 3 images (if regurgitation present)**

- Parasternal long-axis view of the jet without color
- Parasternal long-axis view of the jet with color
- Parasternal long-axis view with continuous wave Doppler

3. ***2-D Parasternal Short axis view of the LV*: At least 1 image**

- Parasternal short axis view at the level of mitral valve leaflet (or young children)
- Parasternal short axis view at the level of the tips of the leaflets (for older children)

4. ***2-D directed M-mode*: At least 1 image**

- M-mode of the LV in short axis view with correct sweep speed and blood pressure stated in the image

5. ***Apical 4 chamber view of the LV* : At least 2 images**

- Apical 4 chamber view without Dual focus
- Apical 4 chamber view with Dual focus

6. ***Pulsed spectral Doppler samples of the mitral valve inflow:* At least 2 images**

- Apical 4 chamber view with pulse wave Doppler inactivated and positioned at the tips of the mitral valve
- Apical 4 chamber view with activated pulse wave Doppler

7. ***Pulsed spectral Doppler samples of the tricuspid valve inflow:* At least 2 images**

- Apical 4 chamber view with pulse wave Doppler inactivated and positioned at the tips of the tricuspid valve
- Apical 4 chamber view with activated pulse wave Doppler

8. ***Continuous wave Doppler sample of the tricuspid valve regurgitation (if any) :*** **At least 2 images**

- Apical 4 chamber view of the TR jet with color only
- Apical 4 chamber view of the TR jet with color and continuous wave Doppler

9. ***Tissue Doppler:*** **At least 6 images**

- Apical 4 chamber view of the left, right and septal ventricular free walls with color only
- Apical 4 chamber view of the left, right and septal ventricular free walls with color flow and spectral mode

10. ***Aortic Doppler:*** **At least 2 images**

- Apical 5 chamber view with color flow only
- Apical 5 chamber view with color flow and spectral Doppler

11. ***Pulsed spectral Doppler samples of pulmonary vein inflow:*** **At least 3 images**

- Apical 4 chamber view with color flow
- Apical 4 chamber view with color flow showing the Pulse wave Doppler position.
- Apical 4 chamber view Pulse Doppler wave forms

12. ***M-mode color-Doppler recording of the LV diastolic flow propagation velocity:*** **At least 2 images**

- Apical 4 chamber view with 2-D color mode and a adjusted color Doppler sector
- Apical 4 chamber view with 2-D color mode, adjusted color Doppler sector and activated m-mode

4.2.8 Pulmonary Evaluations

Spirometry is a valid, reproducible means of monitoring for change in the severity of the respiratory component in lung disease. When properly performed with maximum effort and attention to the details of calibration of the instrument, spirometry maneuvers are among the most reproducible of biomedical measures. Spirometry will be performed in a standardized manner at all sites in accordance with American Thoracic Society (ATS) guidelines, as described in the BH FU II Manual of Operations. The values below will be collected both pre and post bronchodilator. Albuterol will be the preferred bronchodilator unless it is medically contraindicated.

1. FEV1 L
2. FVC L
3. FEV 6 L
4. PEFR L/second
5. Vext L
6. FET 100% seconds
7. FEF25-75 L/second

Percent predicted values for these measures will be calculated at the DCC using the Hankinson and Crapo reference equations.

4.2.9 Stored Blood Samples

We will collect, separate and store components from a five milliliter blood sample from all Follow-up Study II participants at entry into the study and at the end of the study. These samples will be de-identified but linked to the treatment study and Follow-Up I study datasets and will be available to researchers for future studies of sickle cell and related diseases. It is likely that in the future, as with the addition of the cystatin C and urinary microalbumin:creatinine assessments, novel investigations for toxicities or organ function effects of HU will be proposed.

These samples will also be stored indefinitely. Storage of samples with known periods of exposure to HU will be an invaluable resource for these investigations. To this end, Investigators have agreed that saved serum samples will be shipped to a central repository at the NHLBI. Application to the NHLBI repository (BioLINCC) will be made by the DCC and Clinical Investigators to receive review and to seek approval for creation of this biospecimens repository.

4.2.10 Stored Urine Samples

We will collect and store urine at study entry (or within 1 year of study entry) and at exit. The collection of urine samples for future research could help in the discovery of urine biomarkers for renal insufficiency and predictors of kidney disease, as well as the assessment of hydroxyurea metabolites, and determination of differences in renal function preservation between hydroxyurea treated and untreated patients. The urine will be obtained clean catched. The date and time of the urine void and the date and time of the last hydroxyurea dose (if applicable) should be documented. A 10 ml urine sample will be sent to the central lab, transported at 4 °C within 24 hours of collection. It will be divided on receipt into a sample for analysis for microalbumin to creatinine ratio and the remainder will be aliquoted into vacutainer/cryogenic vials containing 1-2 ml of urine each (maximum 5 vials per patient) and frozen at -80°. The samples will be stored at the current central laboratory (Georgia Regents University) in Augusta, GA until submission to the BioLINCC Repository. Application to the NHLBI repository (BioLINCC) will be made by the DCC and Clinical Investigators to receive review and to seek approval for creation of this biospecimens repository.

4.3 STATISTICAL ANALYSES OVERVIEW

With the successful completion of the BABY HUG clinical trial demonstrating the efficacy of HU to reduce the incidence of clinical events which confirms this type of efficacy in older, and younger children with SCA, the statistical focus of the BABY HUG Follow-up Study II will shift to

one of estimation and statistical testing using a variety of different types of HU exposures without strict control of the alpha level for this study. In short, much like R.A. Fisher's recommendation that once an F statistic has been declared statistically significant, one is free to examine the pairwise comparisons with less alpha level control, the BABY HUG investigators will engage in estimating and testing scenarios that preserve the study's power to detect alternatives rather than protecting alpha.

Still, there will be a large number of statistical evaluations performed in this study and it will be important to limit the number of spurious positive associations that may arise during the analyses for this study. Because there is no hypothesized pattern of interest to be seen in the BABY HUG Follow-up Study II, a generalized approach will be formulated that will allow the investigators to describe the changes that occur. The investigators will limit the number of spurious associations by setting the alpha level for determining statistical significance in this study to 0.01 rather than the traditional 0.05 alpha level. This procedure is consistent with the statistical approach used to identify up-regulated genes in micro-array studies, where one seeks to make it "highly likely" that statistically significant results are real by adjusting alpha levels and powers (Benjamini et al., 1995). As an example, among a large group of independent statistical assessments for which 25% of the alternative hypotheses are true and 75% of the null hypotheses are true, setting alpha at 0.01 for each comparison and power to 0.9 for each comparison would mean that on average, 96% of the statistically significant hypotheses declared in the study would come from the 25% of tests where the alternative hypothesis is true. Setting alpha at 0.05 rather than 0.01 would reduce the number of positive predictions to 86%.

A review of the other endpoints and treatment variables to be analyzed shows three different treatment-variable constructs, and four classes of endpoint analyses, to be performed in the BABY HUG Follow-up Study II. As mentioned in Chapter 2, the primary independent variables to be studied in the BABY HUG Follow-up Study II are: 1) the BABY HUG Treatment Study treatment assignment, 2) a variable indicating whether a child has ever received HU

(either as study treatment during the treatment study and/or open-label during follow-up), and 3) a time-dependent covariate indicating HU treatment or no HU treatment over the BABY HUG follow-up interval. The last variable is not a variable per se but a treatment trajectory that will be assessed for its association with SCA-related symptoms and adverse events. The endpoint analyses to be considered in the Follow-up Study II, consist of longitudinal data analyses of continuous endpoints (e.g., CBC results, fetal hemoglobin values, and quantitative spleen and renal evaluations), longitudinal analyses of qualitative evaluations (e.g., spleen activity as evaluated by two masked nuclear medicine physicians), time to event analyses (e.g., the time to the occurrence of adverse events), an analysis of covariance (the analysis of the WPPSI scores), and multivariate analyses (e.g., Vineland Adaptive Behavior Scale).

4.4 STATISTICAL CONSIDERATIONS IN DESIGN AND STUDY SIZE

Summary

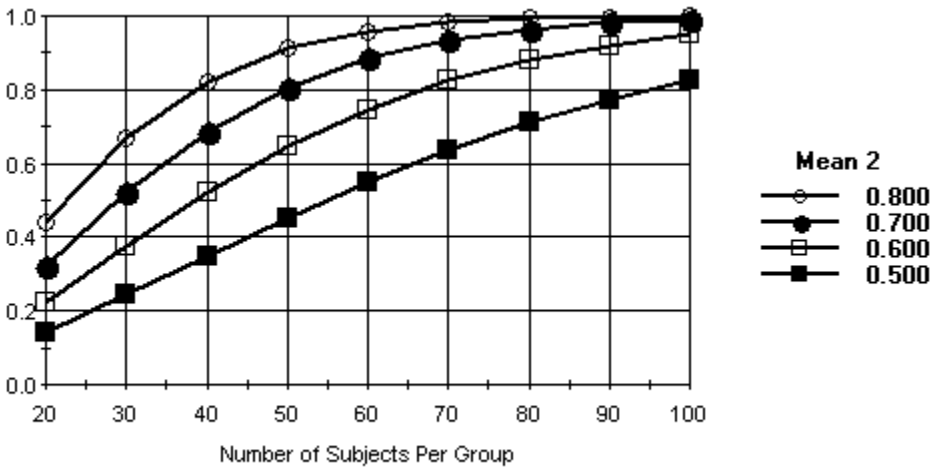
At any point in time, or over a long follow-up period, the power to detect clinical differences with respect to HU treatment will be dependent upon the number of children taking and not taking HU according to the definitions described above. In addition, the number of subjects enrolled in Follow-Up Study II may be unbalanced with respect to the BABY HUG treatment assignments and most children (80%) will likely be taking HU once they are in follow up.

The following Figures can be used to assess the power to detect specified alternative hypotheses for continuous, binary, and time-to-event dependent variables. The operating characteristics of the early versus late comparison with unequal allocation will be similar to the equal allocation scenario so long as the imbalance does not exceed more than a 3 to 1 ratio.

Figures 4.1 to 4.3 show the power of an early versus late comparison for a continuous endpoint being tested at the alpha 0.01 level under a 1:1, 2:1, and 4:1 allocation ratio. These ratios will likely encompass the actual ratio observed at the end of the study.

Figure 4.1

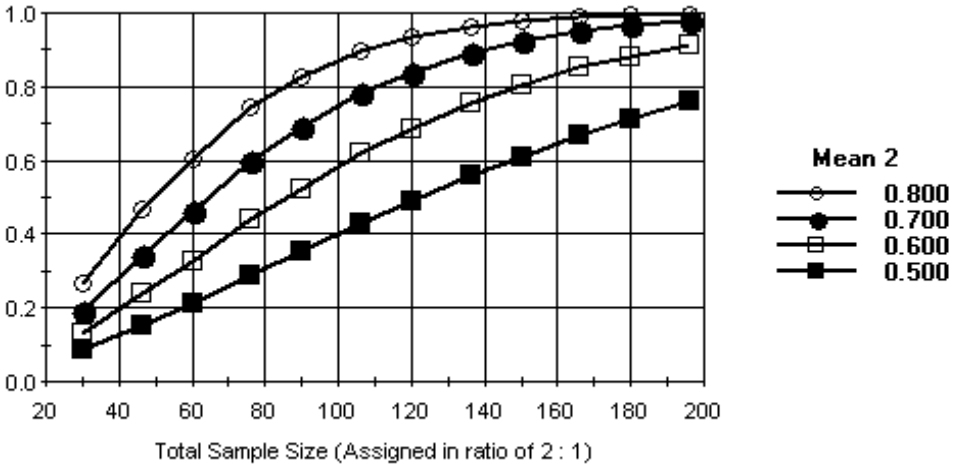
Power to Detect Specified Alternative Effect Sizes (Mean 2)
For a continuous variable when testing an early vs late hypothesis
Equal Allocation



Total Sample Size set at a ratio of 1:1 (HU to PBO). Alpha = 0.010, Tails = 2,
Effect Size (Mean 2) = $((\mu_2 - \mu_1)/\sigma)$

There will be adequate power (greater than 80%) to detect moderate to large effect sizes (greater than 0.6) when testing at the 0.01 alpha level.

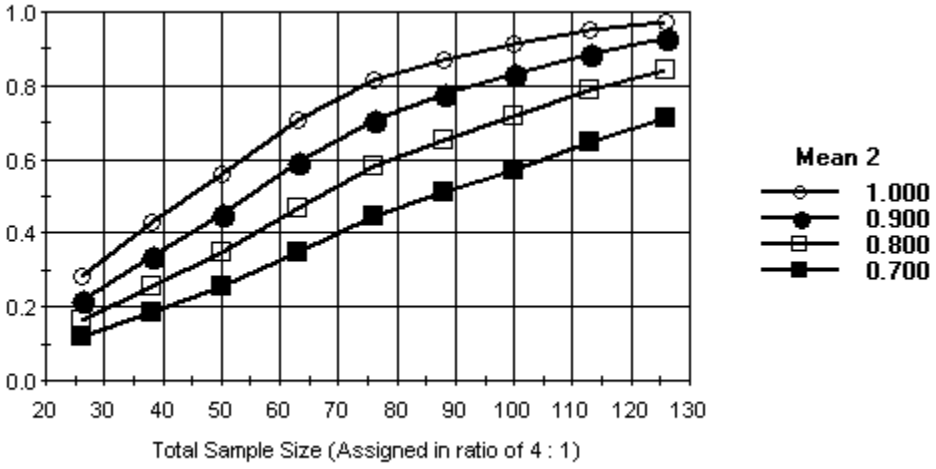
Figure 4.2
Power to Detect Specified Alternative Effect Sizes (Mean 2)
For a continuous variable when testing an early vs late hypothesis
Equal Allocation



Total Sample Size set at a ratio of 2:1 (HU to PBO). Alpha = 0.010, Tails = 2
Effect Size (Mean 2) = $((\mu_2 - \mu_1)/\sigma)$

Figure 4.2 presents the same type of assessment except that allocation has been skewed 2 to 1 in favor of the HU treatment group. Comparison of the two figures shows that there is little difference between the operating characteristics whether or not equal allocation occurs. As long as the elective allocation to late treatment is not severely skewed (greater than 3 to 1 in favor of either late treatment group) adequate power will remain to detect moderate to large effects sizes using the proposed design.

Figure 4.3
Power of the Primary BABY HUG Comparisons
to Detect Specified Alternative Effect Sizes (Mean 2)



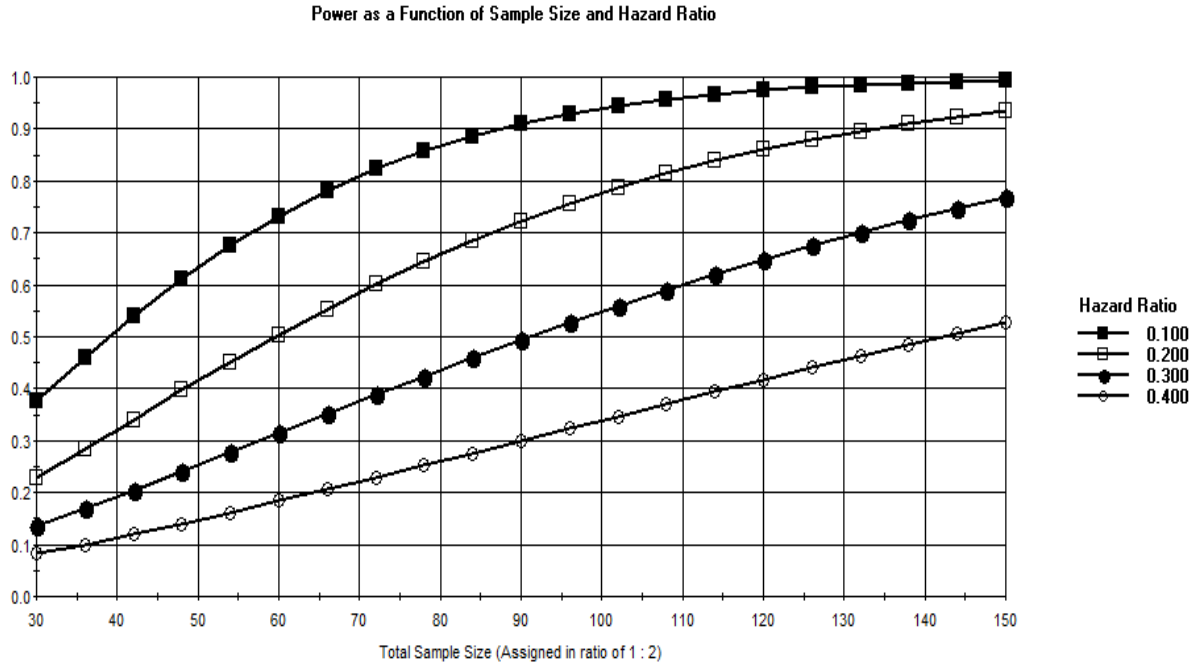
Total Sample Size set at a ratio of 4:1 (HU to PBO).
Alpha = 0.010, Tails = 2, Effect Size (Mean 2) = $((\mu_2 - \mu_1)/\sigma)$

The most extreme imbalance in the comparison groups will likely be when making the comparisons of ever exposed to HU versus never exposed to HU. Preliminary data indicate that there is a two to one ratio of parents/guardians electing to provide their child HU after BABY HUG is completed compared to those who elect not to treat their child with HU. We expect most of the parents/guardians will elect to continue in the follow-up study. If the two to one ratio is applied to both treatment groups, approximately 17% of the BABY HUG population will never be exposed to HU (0.5*0.33 = 0.165) and 83% of the population will be exposed to HU at

sometime during the BABY HUG trial or in the follow-up study. As this is a secondary analysis test, α = 0.01 will be used to declare statistical significance for these comparisons.

Many study endpoints will be represented as time-to-events and will be analyzed using survival analysis methodologies. Here we present a curve to show the power to detect specified alternative hazard ratios (Figure 4.4) as a function of sample size under the assumption that all patients have been recruited by the start of the study, and there are no dropouts over the study period. In general there will be sufficient power to detect large effects sizes (risk ratios of 0.3 to 0.1) with adequate power with a study of 70 to 160 patients with an allocation ratio of 2:1. These are large effect sizes but are consistent with the clinical effects seen for HU in the BABY HUG study.

Figure 4.4
Power of the Primary BABY HUG Comparisons
to Detect Specified Alternative Hazard Ratios
as a Function of Sample Size



Alpha = 0.010, Tails = 2, Accrual Prior, Study Duration = 10,
Drop Rate = None, Hazard Rate = 0.04000

There is no way to adequately measure the power of the proposed comparisons for the time-dependent exposure variable to HU for this study. This would require knowledge about the lagged effects of starting and stopping HU treatment and information about the treatment trajectories for the children. But for a binary covariate, as long as the number having an attribute (such as taking HU at the time) is not too unbalanced, the operating characteristics will not be too different from the operating characteristics for the primary treatment comparisons.

Although not shown here, power curves for binary endpoints show a similar characteristic to those for survival analysis. Large effects sizes (e.g., odds ratios in the range of 2.5 to 5 or more) will be detected with adequate power with the proposed study size.

4.4.1 Data Analysis

4.4.1.1 Introduction

Primary analysis for the BABY HUG Follow-up Study II will focus on estimating differences between patient groups for a variety of different types of endpoints. Assessment of a patient group difference will be based on pooling data across all participating Clinical Centers using all subjects entered. BHFU II analyses will develop statistical models to determine associations and relationships between dependent variables, risk factors and the three treatment variables described earlier in this chapter.

Analysis of binary endpoints will be accomplished using contingency table analysis. Significance of results will be assessed with the Chi-square test uncorrected for continuity or Fisher's exact test. If necessary, contingency table analyses will be adjusted for confounding variables using logistic regression.

For continuous variables, comparisons of groups will be accomplished using Student's t test or the Wilcoxon rank sum test depending on the distributional properties of the data. Stratified designs will be analyzed using regression methods with the strata represented as randomized blocks for the analysis.

Analysis of continuous and categorical endpoints that are measured repeatedly over time, such as weight, height, and CBC measurements will use longitudinal data analysis models (Laird and Ware, 1982; Schlucter, 1992; Liang and Zeger, 1986). Estimation in these models can be in terms of point and interval estimates or trends (i.e., slopes of growth curves) over time.

The comparison between two treatments (or exposures) of time-to-event endpoints will be evaluated using the log-rank statistic. We will use the LIFETEST procedure in SAS to perform the test. The cumulative distributions of this outcome will be estimated using the methods of Kaplan and Meier (Kaplan and Meier, 1958). Multivariate adjustments to this comparison will also be made using the PHREG procedure for SAS to accomplish Cox proportional hazards models analyses (Cox, 1972).

Non-proportionality of the hazards will be investigated by plotting log[-log(S(t))], in which S(t) is the survival function, for important stratifying variables such as age and gender. Should the above functions be non-parallel (and/or cross) for any of the specified variables ($p < 0.01$), the subsequent analysis will be stratified by those variables. Cox proportional hazards models will be stratified for variables that demonstrate non-proportional hazards (crossing or non-crossing). Once determined, we will include these variables as stratification variables in the Cox regression. Analyses for the regressors will be summarized across the strata. Subjects who are lost to follow-up before the end of the follow-up interval will be censored at the time of their last visit. For events that can occur more than once in a child, the counting processes extension of the Cox model in SAS will be used to measure the relative impact of treating subjects with HU on these endpoints (Andersen and Gill, 1982).

4.4.1.2 Regression Analyses and Adjustment

We will adjust study results for potential confounding factors in secondary analyses. The addition of confounding variables generally improves the operating characteristics of analyses of the main effect, but given the small study size, the number of confounding variables to be

considered in any secondary analysis will be small (limited to five or ten). We will use step-wise regression methods to isolate and include the most important confounding variables in the regression model.

Tests of interaction will be part of regression analyses. Most of these analyses will be designed to determine if BABY HUG Treatment Study treatment effects or other HU exposure effects are consistently observed across different clinical groups of subjects. If an interaction test is significant ($p < 0.01$), we will report that treatment effects differed according to the stratification dictated by the interaction test. Interaction tests such as these have low power to detect specified alternatives (e.g., half the efficiency of a main effects comparison). Additional analyses will be required to support the discovery of a proposed interaction, as a large number of interaction tests will be performed and some, by chance, will be found to be significant. One important analysis will be to incorporate variables that measure the duration of HU open-label treatment over the follow-up interval. This will be done by including variables that measure the duration of use for a cross-sectional analysis or time-dependent covariates for a longitudinal analysis.

We will use SAS procedures to perform adjusted analyses, PROC GLM to perform randomized block analysis of variance, PHGLM to perform stratified and standard Cox proportional hazards analyses, PROC GENMOD and MIXED to perform longitudinal data analyses, and PROC LOGISTIC to perform unconditional logistic regression. The standard output from these procedures provides point estimates for the regression coefficients, standard error estimates and confidence intervals. The results of these analyses are printed into computer files so that they can be directly inserted in progress reports using PROC REPORT. In some instances, the procedures in SAS will not suffice since SAS procedures usually do not include methods to incorporate information about missing data, nor do they include complex models specifically designed to relate a biological process with the risk of disease progression. We will use PROC IML and PROC NLIN to program the required models if necessary.

4.4.1.3 Longitudinal Data Analysis

Many of the endpoints that will be collected and analyzed in the BABY HUG Follow-up Study II will be longitudinal in nature with collection points at entry into the treatment study, two years after the child is enrolled into the treatment study (exit), two years after enrollment into Follow-Up Study I (active group only), and five years or exit from Follow-Up Study II (whichever comes first). Examples of such endpoints are: HJB, pit counts, GFR estimates, liver-spleen scan data, anthropometry, TCD velocity, VDJ recombination events, and neuropsychology testing. For each of these endpoints, we will present box plots of the measures according to the three different treatment indicators (BABY HUG Treatment Study assignment to HU, ever treated with HU (see page 4-1), and a time-dependent indicator of HU treatment) at each collection point. For the longitudinal analysis, we will use the baseline collection from the treatment study as an adjustment variable and then test the longitudinal trajectory for differences according to the treatment variable being studied and time-by-treatment interactions. Time trajectories will be analyzed using linear, quadratic and cubic terms. All data will be analyzed using generalized estimating equations (GEE) or mixed model analysis of variance; binary data will be analyzed in GEE using a logit link function and continuous data will be analyzed using the linear link function.

4.4.1.4 Missing Data Analysis

We will generally use the methods of Rubin (Little and Rubin, 1987) to impute missing data from subject's records with complete data to complete the records for subjects with missing data. This method has been accepted by the FDA as a legitimate method for correcting for missing data. To assess the impact of missing data on our analyses, we will repeat each missing data analysis without imputation and compare the inferential results. If the results are similar, we will report that standard analyses and comment in the paper that accounting for missing data did not change the results. If the inferences do change, the missing data results will be placed in the paper and we will add commentary about the risks and benefits of missing

data analysis in the discussion. We will also use analyses involving rank statistics in which subjects who die or have bad clinical events are given the worst rank for other dependent variables. This technique has been used successfully in the Multicenter Study of Hydroxyurea (McMahon et al, 1997). For categorical data or time to event data, the composite endpoint of death or the event (such as occurrence of acute chest syndrome) can be used.

4.4.2 Interim Monitoring

During the course of this study, the BABY HUG OSMB will meet semi-annually to review interim data analyses to monitor subject safety and advise the trial sponsor, NHLBI on their findings, recommendations and the overall progress of the study. These interim OSMB analysis reports for the OSMB will include, but are not limited to comparisons by treatment group for:

1. Child characteristics at baseline, completion of the BABY HUG Treatment Study, four years after randomization into the BABY HUG Treatment Study (two years on the Follow-up Study I), and in the Follow-up Study II at age 10 Additional measurements of specific endpoint data will be taken annually in the active group (see Appendix A):

   Spleen function

   Spleen size

   Pit counts

   HJB quantitation

   Schwartz equation GFR estimates

   Cystatin C GFR estimate

   Urine microalbumin:creatinine ratio

   Neuropsychological Testing Performance

   Height and weight

TCD measurements

Cardiac Echocardiography Results with Brain Natriuretic Peptide Testing

MRI/MRA

Pulmonary Function Testing Performance

Blood count toxicities

1. HU dose tolerated or maximum HU dose tolerated (mg/kg)
2. Safety assessments and clinical events
3. Serious Adverse Events
4. Distribution of baseline characteristics:

   Gender

   Age at Entry

The OSMB will also review Clinical Center study performance including completeness of follow-up, submission of forms and quality of laboratory data submitted, number of subjects lost to follow-up, and protocol violations.

4.4.3 Safety Related Outcomes

All clinical data will be collected retrospectively every six months. For subjects who agree to active follow-up, the results of the active assessment studies will be reported as soon as they are performed. Serious adverse event (SAE) reporting for the conditions listed in Table 4.1 will include only events that occur during the first five days following performance of the active assessment studies. Events defined as “major” events will be reported to the DCC immediately for all enrolled subjects. See Section 4.4.3.1 “Major” Events for description. Otherwise, a structured listing of clinical events will be submitted to the DCC and tabulated based on the affected organ system according to standardized monitoring procedures (e.g., MedDRA). These will be tabulated and a systematic review will be made to determine if one treatment group (or HU exposure type) has more reports of SAEs than the other treatment

group (or HU exposure type). Depending on the evidence accumulated, it will be the responsibility of the OSMB Chair, the Executive Secretary of OSMB and the Project Officer to decide whether a full meeting of the OSMB is necessary to discuss the results and make recommendations, whether a conference call is necessary, or whether the report warrants no further action. Classification and reporting considerations are discussed in Section 9.2.4 of this protocol.

Ninety-five percent confidence intervals for the difference between proportions for the different treatment groups will be used to compare the occurrence of SAEs. If the confidence interval for the difference in these proportions does not cover zero, the Project Officer will be notified promptly. The Executive Secretary of the OSMB and the Project Officer in consultation with the OSMB Chair will then recommend whether there should be an emergency meeting (or conference call of the OSMB) to determine the appropriate actions for this study.

Table 4.1

Definition of Serious Adverse Events

A **serious adverse event** is any one of the following.

1. Death
2. Life-threatening event
3. Prolonged hospitalization (greater than 7 days)
4. Splenic sequestration crisis
5. Stroke, TIA
6. Acute chest syndrome
7. ICU admissions

Serious Adverse Events that are SCA-related have been added to the list, as defined by the FDA. Item #3 has been modified from the FDA definition because frequent hospitalizations occur as a consequence of having sickle cell disease without being enrolled in a study.

4.4.3.1 "Major" Events

In addition to reporting serious adverse events, the NHLBI requires that all "major" events be reported to the Data Coordinating Center (DCC) as soon as Clinical Center Staff become aware of the event. The NHLBI has defined a "major" event as an event which would meet the definition of a reportable serious adverse event as described in Table 4.1, but which does not occur following the performance of a BHFU II active assessment study (e.g. liver/spleen scan, abdominal sonogram, MRI/MRA, cardiac echocardiogram, neuropsychology test). The reported information will be communicated to the Observational Study Monitoring Board (OSMB) Chairman for informational purposes and comment, whereas Serious Adverse Events will continue to be reported to the FDA under the BABY HUG IND as well as to the OSMB. All safety reporting to the IND will comply with 21 CFR 312.32 and as updated by the Final Rule: Investigational New Drug Safety Reporting Requirements for Human Drug and Biological Products and Safety Reporting Requirements for Bioavailability and Bioequivalence Studies in Humans (2010).

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY II PROTOCOL

CHAPTER 5

OPEN-LABEL TREATMENT WITH HYDROXYUREA

5.1 OVERVIEW

Treatment with open-label HU during Follow-Up Study II is at the discretion of the parent or guardian after consultation with the child's hematologist. Regardless of the follow-up group in which the family chooses to have their child participate, all subjects on open-label HU will be followed according to local center hydroxyurea standards to monitor for toxicity. All blood tests performed for this purpose will be collected and processed locally, except for those noted as "core" measurements in Appendix A. For use of open-label HU, the dose, formulation, monitoring intervals, and toxicity levels stated below are guidelines only. Actual treatment is in accordance with routine local clinical care. Cost of treatment and monitoring will be borne by the family and their child's insurer.

5.2 DOSE TITRATION OF OPEN- LABEL HYDROXYUREA

The use of open-label HU will not be specifically regulated as was done in the BABY HUG Treatment Study. The dose of HU prescribed will be carefully recorded and reported semi-annually. Local criteria for dosing, dose escalation, and toxicity values will be used. The following guidelines for local adaptation are suggested:

- Subjects should begin open-label treatment with HU at the same dose as the dose of the BABY HUG Treatment Study drug which was 20 mg/kg. However, if a subject's maximum tolerated dose was determined during BHFU I, then he/she should remain on that dose.

- Dose escalation of 5 mg/kg every 6-8 weeks should be strongly considered if there is no toxicity to a maximum tolerated dose or 35 mg/kg.
- A CBC with differential white blood cell count and reticulocyte count should be monitored monthly while on HU.
- Predetermined toxicity levels should be utilized to monitor blood counts. The toxicity values should be no lower than an ANC<1250/mm^3, platelet count <80,000/ mm^3, a hemoglobin level below 6 gm/dL or greater than 20% fall in hemoglobin concentration from a three-month rolling average. Local clinical criteria with higher cut off points for declaring a toxic value may be used if desired.
- Dose should be reduced for severe or recurrent toxicities, with an attempt to re-escalate the dose if six months pass without subsequent toxicity.

5.3 MONITORING FOR TOXICITY

All laboratory data as well as the labs done for each study visit will be reviewed and determination of toxicities will be made by the local Clinical Center BABY HUG PI and appropriate other clinical staff at each Clinical Center according to local Clinical Center practices and standards. The results will be reported retrospectively every six months by structured abstraction of the medical record. The PI, nurse coordinator and all clinical staff may view all of the laboratory data from each study visit.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY II PROTOCOL

CHAPTER 6

LABORATORY ANALYSES AND SPECIMENS

6.1 OVERVIEW

All clinical hematology, LDH, bilirubin, ALT and urine osmolality specimens will be processed locally. Core laboratories will process specimens and report the test result and normal laboratory levels for fetal hemoglobin, creatinine and BUN, pit count, HJB, VDJ, microalbumin:creatinine ratio-urine, and cystatin C as described in the BABY HUG Follow-up Study II Manual of Operations. The TCD Core Laboratory will review a clinical TCD performed at approximately 10 years of age in the passive group. In the active group, a study-sponsored TCD will be performed by the Clinical Centers at approximately 10 years old and sent to the TCD Core Laboratory for interpretation. The NHLBI Specimen Repository will be utilized for processing study specimens if the Repository approves receipt of the specimens. The amount of blood and times of collection are specified in Appendix B.

6.2 PITTED CELL CORE LABORATORY

For all participants, a pit count will be obtained using one drop of blood preserved in glutaraldehyde at study entry and exit. An additional sample will be obtained for subjects in active follow-up once every year. Specimens will be stored and refrigerated locally, and shipped in batches to the Pitted Cell Core Laboratory at UT Southwestern-Children's Medical Center Dallas Clinical Laboratory. The technique for sample collection is described in the MOO for the BABY HUG Treatment Study and the BABY HUG Treatment Study Protocol.

6.3 CYSTATIN C CORE LABORATORY

Specimens for measurement of cystatin C will be processed in the same fashion as described in Section 9.11 of the BABY HUG Treatment Study Protocol.

6.4 VDJ/DNA EXTRACTION/STORAGE CORE LABORATORY

At entry and exit of the Follow-Up Study II, DNA will be isolated using a standard commercially available kit (Puregene DNA Isolation Kit, Gentra Systems Inc.). The purified DNA will be quantitated using a spectrophotometer and used directly in the VDJ mutation assay. The overall goal of the VDJ studies is the investigation and quantification of the mutagenic and carcinogenic risks of HU therapy for very young children with SCA enrolled in the BABY HUG study. To accomplish this goal, we will analyze peripheral blood for the presence of changes in chromosomal integrity that indicate unrepaired genetic damage. The specific aims of the VDJ mutation study are: to quantitate the frequency of "illegitimate" VDJ recombination events that occur between the T cell receptor gamma (TCD-gamma) and beta (TCR-beta) gene loci located on chromosome 7; and to compare using serial measurements the frequency of VDJ mutational events among subjects depending on their time of HU exposure (treatment study, Follow-up Study I, Follow-up Study II, never).

6.5 HJB CORE LABORATORY

At entry and exit of the Follow-Up Study II, a sample will be collected and processed in the same fashion described in section 9.4 of the BABY HUG Treatment Study Protocol. An additional sample will be obtained for subjects in the "active" group once every year. A small aliquot of RBC will be fixed in ice-cold methanol and frozen at -85^{o}C according to a previously published protocol. This sample will be shipped frozen to Litron Laboratories, Inc. in Rochester, NY and analyzed by flow cytometry for quantitation of HJB (micronuclei) in both immature and mature erythrocytes. As above, serial measurements of the quantitative HJB measurement will be compared amongst subject groups depending on their time of HU exposure (treatment study, Follow-up Study I, Follow-up Study II, never).

6.6 NHLBI SPECIMEN REPOSITORY

If permission is granted by the parent/guardian, five milliliters of blood will be collected at entry and exit of the Follow-up Study II for future use by BABY HUG and other sickle cell

disease investigators. The sample will be divided into serum, plasma and cell pellet aliquots and stored at the Georgia Regents University. In addition, this laboratory will conserve residual plasma, serum, and cell pellets. At the end of the BABY HUG Follow-up Study II, these specimens will be shipped to the NHLBI Specimen Repository (BioLINCC - Biologic Specimen and Data Repository Information Coordinating Center) provided informed consent has allowed for their long-term storage and use **and** the application submitted by the investigators for use of the NHLBI Specimen Repository is approved. At the end of Follow-Up Study II, a public-use database will be delivered to the NHLBI that will allow clinical data to be linked to the specimens to aid investigators in carrying out future SCA-related studies. If families give consent, DNA will be extracted and saved. De-identified specimens will not provide any identifier to the subject from whom the specimen(s) was (were) obtained.

6.6.1 Specimen Storage

Parents/guardians must provide consent to have their child's specimens stored in a central repository. The planned specimens are outlined in Appendix A. Specimens will be prepared as outlined in the BABY HUG Follow-up Study II Manual of Operations. Labels, provided by the DCC, will be applied to the specimens that will be stored at -80 and then sent to the NHLBI repository. Labels will be coded so that the specimens can be linked to relevant clinical data (e.g. clinical events, liver/spleen scan results, etc) in the future.

6.6.2 Obtaining Stored Specimens

The process involved in requesting biospecimens is determined by the "Proprietary Period" or "Open Period" status of the study collection. The "Proprietary Period" lasts until the clinical study data are made available for sharing following the NHLBI Limited Access Data Sharing Policy timeline. During the "Proprietary Period" only centers involved in the BABY HUG Follow-up Study II will be permitted access to the specimens. During the "Open Period"

specimens will be available to all investigators successfully completing the application process as described below.

Investigators will need to provide a design of the proposed research and evidence of the qualification to perform the research. In addition, evidence of the availability of funding to perform the requested research must also be provided. Furthermore, investigators wishing to obtain specimens must address ethical and legal considerations, including consistency with the terms of the informed consent and compliance with human subjects and HIPAA regulations. Investigators requesting biospecimens during the "Proprietary Period" will need permission of the BABY HUG Follow-Up Study II Steering Committee prior to obtaining the specimens.

6.7 HEMOGLOBIN F, BUN AND MICROALBUMIN: CREATININE RATIO CORE LABORATORY

A 0.5 mL blood sample and a 10.0 mL urine sample will be collected at each Clinical Center and shipped to the Georgia Regents University a core laboratory. Georgia Regents University will analyze the blood samples to provide HbF, creatinine, and BUN measurements. Georgia Regents University will also analyze the urine sample to supply the microalbumin : creatinine ratio. The samples will be stored at the current central laboratory (Georgia Regents University) in Augusta, GA until submission to the BioLINCC Repository.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
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CHAPTER 7

CHAPTER 7GUIDELINES FOR STANDARD CLINICAL CARE

7.1 INTRODUCTION

The basic principles of supportive care for young subjects enrolled in Follow-Up Study II are similar to those in the BABY HUG Treatment Study. The cooperation of all medical staff involved in the clinical care of study subjects will be solicited to enhance adherence to the Follow-up Study II protocol. Parent education and guidelines for the diagnosis and treatment of common clinical events are addressed in the BABY HUG Treatment Study Protocol Section 8.4. At no time should the performance of the BABY HUG Follow-Up Study II protocol be allowed to compromise the elements of good clinical care of the subjects enrolled in the study.

7.2 IMMUNIZATIONS

All routine pediatric immunizations should be given as per standard clinical recommendations as noted in the BABY HUG Treatment Study Protocol Section 8.2 and in accordance with local routine clinical care. (American Academy of *Pediatrics Red Book 2009*)

7.3 PROPHYLACTIC MEDICATIONS

Twice daily prophylactic penicillin should be continued until at least five years of age. The dose, formulation and use of an alternative antibiotic are in accordance with routine local clinical care. Reminders about the need for this prophylactic agent should be offered at each clinical contact.

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CHAPTER 8

SPECIAL STUDIES AND READING GROUPS

8.1 INTRODUCTION

Special studies and event reports that will be centrally evaluated by individuals independent of the BABY HUG Clinical Centers include: liver-spleen scans, abdominal ultrasound, MRI/MRA, TCD, and Cardiac Echocardiograms.

8.2 LIVER-SPLEEN SCANS

Tc99m sulfur colloid liver-spleen scans will be performed once, only in subjects whose parents/guardians gave consent to active assessment, at 10 years of age according to standard techniques. The results of this scan will be compared to the scans performed at screening and completion of treatment in the BABY HUG Treatment Study and those performed at two years after exit from the BABY HUG Treatment Study (during Follow-up Study I). Results will be assessed by a panel of three pediatric radiologists who are unaware of the original treatment assignment of the child. The reading process has previously been described in Section 9.5 of the BABY HUG Treatment Study Protocol. The proportion of subjects in each treatment group over the nine years of combined treatment and follow-up will be compared according to spleen function (normal, decreased, or absent). In addition, the number of times that there is a decline in splenic function from one category to another will be compared in each treatment group. Scans that demonstrate an improvement in uptake will be scored as not demonstrating a decline.

8.3 PITTED CELL COUNTS (Pit Counts) and HOWELL JOLLY BODIES (HJB)

Pit counts will continue to be done in a single laboratory, the Pitted Cell Core Laboratory at the University of Texas/Children's Medical Center Dallas. Tubes containing the glutaraldehyde buffer and directions for specimen collection will be provided to the Clinical

Centers by the Pitted Cell Core Laboratory as in the MOO of the treatment study. HJB determinations will be performed in the single laboratory used in the BABY HUG Treatment Study. Pitted cell counts and HJB will be performed from specimens collected from all Follow-Up Study II subjects twice, at entry and exit in the BABY HUG Follow-up Study II. Subjects whose parents/guardians gave consent for active follow-up will have additional measurements carried out every year after entry into Follow-Up Study II.

8.4 CLINICAL EVENTS

Clinical events (hospitalizations and specific other sickle cell related events) will be retrospectively abstracted from the medical records of study participants at specified intervals (two times per year). Clinical Centers will be asked to classify the event based upon BABY HUG criteria using simple yes/no data forms. Definitions of clinical events can be found in Appendix F of the BABY HUG Treatment Study Protocol except for the post-hoc Steering Committee definition of splenic sequestration which is defined as follows:

> *Splenic Sequestration:* 2 cm or more increase from last visit in palpable spleen size AND a decrease in Hb of 2 g/dL or more below the last steady state value as determined by the investigator.

8.5 CREATININE CLEARANCE AND CYSTATIN C

Creatinine clearance will be estimated from cystatin C measurements and the Schwartz equations as indicated in the BABY HUG Treatment Study MOO. These studies will be done at the times indicated in Appendix A.

8.6 ABDOMINAL ULTRASOUND

An abdominal ultrasound will be performed once, only in subjects whose parents/guardians gave consent to active assessment, by standard clinical techniques to estimate the size of the spleen and the presence of gall stones according to the techniques outlined in the MOO of the BABY HUG Treatment Study. This assessment will document if the spleen is enlarged or if it has involuted. The presence or absence of gall stones is a secondary

endpoint assessment for the degree of hemolysis occurring over time. Kidney length and volume will be assessed, as well as evaluation for evidence of renal disease and infarcts.

8.7 TRANSCRANIAL DOPPLER (TCD)

Transcranial Doppler studies will be performed by the Clinical Centers as part of routine clinical care and one TCD exam will be interpreted by the TCD Core Laboratory for all participants. As annual TCD evaluation is standard of care in the BABY HUG Follow-up Study II age group, it is anticipated that most subjects will have at least one TCD study during the follow-up period. In the active group, one study-sponsored TCD exam will be performed and interpreted prior to exit from the Follow-Up Study II at approximately 10 years of age. The studies will be performed by technicians certified by the Medical University of South Carolina (MUSC) and the Medical College of Georgia (MCG) for the BABY HUG Treatment Study. The study performed for clinical indications (the one closest to the age of 10) or as part of the study, scheduled at 10 years of age, will be copied and sent for review and analysis by the TCD reading committee.

8.8 ECHOCARDIOGRAM/BRAIN NATRIURETIC PEPTIDE TESTING

An echocardiogram using standard clinical techniques will be performed to detect structural abnormalities of the heart. These abnormalities include the size of the heart chambers, the thickness of the walls of the chambers, blood flow through the heart valves, and an estimate of the tricuspid regurgitation velocity (TRV). A central reading of all echocardiograms will be performed. The echocardiographic examination will be performed at each Clinical Center using a high-resolution commercially available cardiac scanner with transducer frequency and focus appropriate for the patient's size and body habitus. The echocardiographic examination includes complete imaging sweeps from the subxiphoid, apical, parasternal and suprasternal notch views in the long- and short-axis views as well as angled views to optimally visualize cardiac and vascular structures. The atrioventricular and semilunar valves, branch pulmonary arteries, atrial and ventricular septum and aortic arch will be

interrogated by color coded and pulsed Doppler to evaluate for valvular stenosis or regurgitation, vessel stenosis, intracardiac thrombi, and regional wall motion abnormalities. Full anatomic evaluation for congenital heart disease is conducted by two-dimensional and Doppler echocardiogram. Brain Natriuretic Peptide testing should be performed and tested locally in an ACP certified laboratory at the same time as the echocardiogram.

8.9 PULMONARY FUNCTION TESTS (PFTs)

A standard set of PFTs including Spirometry (pre- and post-bronchodilator response) and Diffusion Capacity (DLCO) using standard clinical techniques will be performed to assess lung function at age 10. A central review of all PFT results will be performed.

8.10 MAGNETIC RESONANCE IMAGING AND MAGNETIC RESONANCE ANGIOGRAPHY (MRI/MRA)

MRI/MRA will be performed without sedation using standard clinical techniques. Should a Clinical Center encounter difficulty in performing an MRI/MRA due to a subject's inability to remain still, they will be required to request a Protocol Exception to use sedation. This will require Steering Committee and Clinical Center IRB approval and additional consent from the parent/guardian prior to performance of the studies. All MRI/MRAs of the brain will be centrally read for final classification of abnormalities and review of measurements.

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FOLLOW-UP OBSERVATIONAL STUDY II PROTOCOL

CHAPTER 9

FOLLOW-UP PROCEDURES

9.1 INTRODUCTION

Subjects in both the passive and active follow-up groups will have data from clinic visits no less than every six months in the five years of Follow-Up Study II until the common termination date, December 2016, is reached. The first subject was enrolled into the BABY HUG Treatment study in October 2003 and the last subject was enrolled in September 2007. Therefore, the total follow-up period including randomized treatment time will range from 9 years and 3 months to 13 years and 2 months.

9.2 FOLLOW-UP VISITS

Once informed consent for Follow-Up Study II is obtained, retrospective data abstraction will be carried out for all medical visits that occur between Follow-Up Study I and enrollment in the Follow-Up Study II. The type of data to be collected and recorded on structured reporting forms includes (but is not limited to) results of physical examinations, interim history assessments, hospital/Emergency department visits and local laboratory assessments.

All Follow-Up Study II visits will be performed at the Sickle Cell or outpatient clinic of the local Clinical Center and subjects will be evaluated in accordance with routine local clinical care. At each visit during this period the evaluation for those subjects on HU will include an interval medical history and information regarding adverse events and toxicity. In addition, height and weight will be recorded. Results of all testing obtained for clinical indications, including but not limited to TCDs, neuroimaging, and medical consultations, will be collected in a systematic manner in the Follow-up Study II.

The data abstraction forms will be due on January 1 and July 1 of each study year, with the initial form being due on July 1, 2012.

9.2.1 Passive Follow-up Group

All subjects in the passive group of the Follow-up Study II will have cystatin C, BUN, Creatinine, HbF, pit counts, HJB, VDJ, urine microalbumin:creatinine ratio and a stored blood sample collected at entry and exit to Follow-Up Study II. These samples will be shipped to specified central laboratories for analysis (see Appendix A). CBCs, reticulocytes, differential, LDH, bilirubin and ALTs will be collected at entry and exit and tested at the Clinical Center local laboratory. All other laboratory testing will be limited to those tests performed in accordance with routine local clinical care, including HU monitoring for those subjects being treated with HU. Study data will be abstracted from the medical record using structured forms and collected semi-annually. Parents/guardians will be presented with a questionnaire regarding the subject's health status related to enuresis, snoring/obstructive sleep apnea and priapism once every year.

Subjects in the passive group may also have additional testing performed for clinical reasons, including liver/spleen scan, abdominal sonogram, pulmonary function testing, MRI/MRA, cardiac echocardiogram, or neuropsychology testing. Should this testing occur, the locally generated report and images will be centrally read just as for the study-related imaging procedures. If more than one of any of the above mentioned procedures is performed, then the one which is performed closest to 10 years of age should be provided to the DCC for central review.

9.2.2 Active Follow-up Group

Those subjects in the active group will have all of the same testing as in the passive group, as described in Section 9.2.1, but will also have HbF, pit counts, HJB, VDJ, cystatin C, Creatinine and BUN, CBC, reticulocytes, differential, LDH, bilirubin and ALTs performed every year (see Appendix A). Active follow-up will also involve the collection of many of the same primary and secondary endpoint measurements as in the BABY HUG Treatment Study. These will include: Hgb F, pit counts, HJB, liver-spleen scan information (quantitative and qualitative),

multi-digit serum creatinine, BUN (for the calculation of the Schwartz estimates of GFR), cystatin C, and neuropsychological testing. Additional testing in the active group will include cardiac echocardiography with BNP, pulmonary function testing and MRI/MRA. The schedule of subject visits during which specimens will be collected for laboratory testing and imaging studies is shown in Appendix A. The timing of the active follow-up group special studies will be based on the subject's age. The ideal time window is at **10** years of age. This schedule is designed to measure all endpoints along the combined continuum of treatment and follow-up.

9.2.3 Collection of Laboratory Data

For all study visits, any local Clinical Center staff member may collect laboratory data. However, staff must be study certified for access to the BHFU II Study database for data entry (as described in the BHFU II Manual of Operations).

9.2.4 Adverse Event Reporting

Clinical events reported on the semi-annual follow-up form (Form 10) will be tabulated by the DCC based on the affected organ system. If any child dies while on study, efforts will be made to obtain complete post-mortem information. Narratives of fatal events will be sent with study forms to the DCC. As noted in Chapter 4, all safety reporting to the IND will comply with 21 CFR 312.32 and as updated by the Final Rule: Investigational New Drug Safety Reporting Requirements for Human Drug and Biological Products and Safety Reporting Requirements for Bioavailability and Bioequivalence Studies in Humans (2010).

The NHLBI OSMB reviews the protocol and amendments. A progress report showing results according to the different treatment types (see Chapter 4) will be forwarded by the DCC to the NHLBI and the OSMB at these times and their recommendations will be expeditiously implemented. The OSMB members will also be provided with annual reports documenting each child's growth, development and progress and other analyses as requested. The OSMB will be informed of all serious and major adverse events as soon as they occur, as outlined in Chapter

4. The OSMB may recommend early termination of the study to the NHLBI for considerations of safety**.**

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY II PROTOCOL

CHAPTER 10

CLOSE-OUT PROCEDURES

10.1 OVERVIEW

As noted in Section 9.1, the anticipated common termination date for this proposal is December 31, 2016. As mentioned in Chapter 2, regardless of efficacy outcome it is important to determine a complete long-term toxicity profile for the subjects in the BABY HUG Treatment Study. If HU treatment is found to be efficacious, it will be essential to determine how long this effect is preserved.

10.2 DEBRIEFING CONTACT

After final long-term follow-up data have been collected and final reports on the results have been prepared for presentation or submitted for publication, each child's family will be scheduled for a debriefing contact. Families will be informed of the results of the Follow-up Study II and any recommendations of the investigators.

10.3 FINAL STUDY DATA AND DISSEMINATION OF RESULTS

Data processing and analysis of final Follow-up Study II data will proceed on a "time-is-of-the-essence" basis. Clinical Centers will implement the following procedures for finalization of study data. All queries for data clean-up including resolution of forms/procedures expected but not completed, as determined by the DCC, will be addressed within two months of the last follow-up visit. Clinical Centers will be responsible for archiving records that document reported events and specified outcomes. The DCC will archive all electronic study data. Data from the Core Laboratories, Endpoints Evaluation Committees and medical records serve as the definitive sources for subject outcomes in the study.

The OSMB will review the final data analyses regarding the main findings of the study, including analyses for efficacy and safety, at a planned final meeting. These data analyses will

form the basis of the final consensus recommendations from the OSMB, Steering Committee and the NHLBI. These consensus recommendations will be shared first with the study subjects' families and will be made public as soon as possible thereafter. The final data analysis report will be available for submission to the FDA and for archival as will any databank studies.

Archiving of central source data, including Core Laboratory results, will be consistent with requirements for a study conducted under an Investigational New Drug (IND) Exemption and sponsored by the NHLBI and NICHD. Storage of frozen and preserved specimens will be maintained according to the requirements of the NHLBI specimen repository as outlined in Chapter 6, following approval of the application to establish a biorepository. The DCC will archive study data in accordance with FDA guidance and NHLBI requirements. Public data files will be made available according to NHLBI policy. The study will be registered at ClinicalTrials.gov.

PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL (BABY HUG)
FOLLOW-UP OBSERVATIONAL STUDY II PROTOCOL

CHAPTER 11

ORGANIZATIONAL STRUCTURE AND PARTICIPATING UNITS

11.1 INTRODUCTION

The BABY HUG Follow-Up Study II will be conducted by the Clinical Centers who successfully competed in the peer reviewed responses to the contract solicitation. The Pharmacy Distribution Center and Clinical Center Pharmacies from the Baby HUG interventional trial are no longer necessary. All open-label HU will be prepared and distributed by the pharmacy at each Clinical Center or at another commercial pharmacy. Descriptions of the various participating units can be found in Chapter 13, Section 2, of the BABY HUG Treatment Study Protocol.

Administration of this study will involve the same structure, personnel, and reporting procedures as in the BABY HUG Treatment Study. Descriptions of these functions can be found in Chapter 13, Section 3, of the BABY HUG Treatment Study Protocol. Exhibit 11.1 details the organizational chart for the Follow-up Study II. A list of participating centers is found in Exhibit 11.2.

11.2 Clinical Center Staff

The Clinical Center staff will be trained in accordance with the procedures set out in the Follow-Up Study II protocol, many of which are the same as those in the BABY HUG Treatment Study and Follow-up Study I protocols. The objective is to standardize all study procedures carried out in the Clinical Centers. Treatment of study subjects whether on open-label HU or not, will be in accordance with routine local clinical care.

11.3 Monitoring and Endpoint Evaluation

Study monitoring will be carried out by the OSMB and Steering Committee based on this protocol. The Endpoints Evaluation Committees will perform their respective functions according to the BABY HUG Treatment Study Protocol.

Exhibit 11.1
Pediatric Hydroxyurea Phase Clinical Trial (BABY HUG)
Follow-up Observational Study II Organizational Chart

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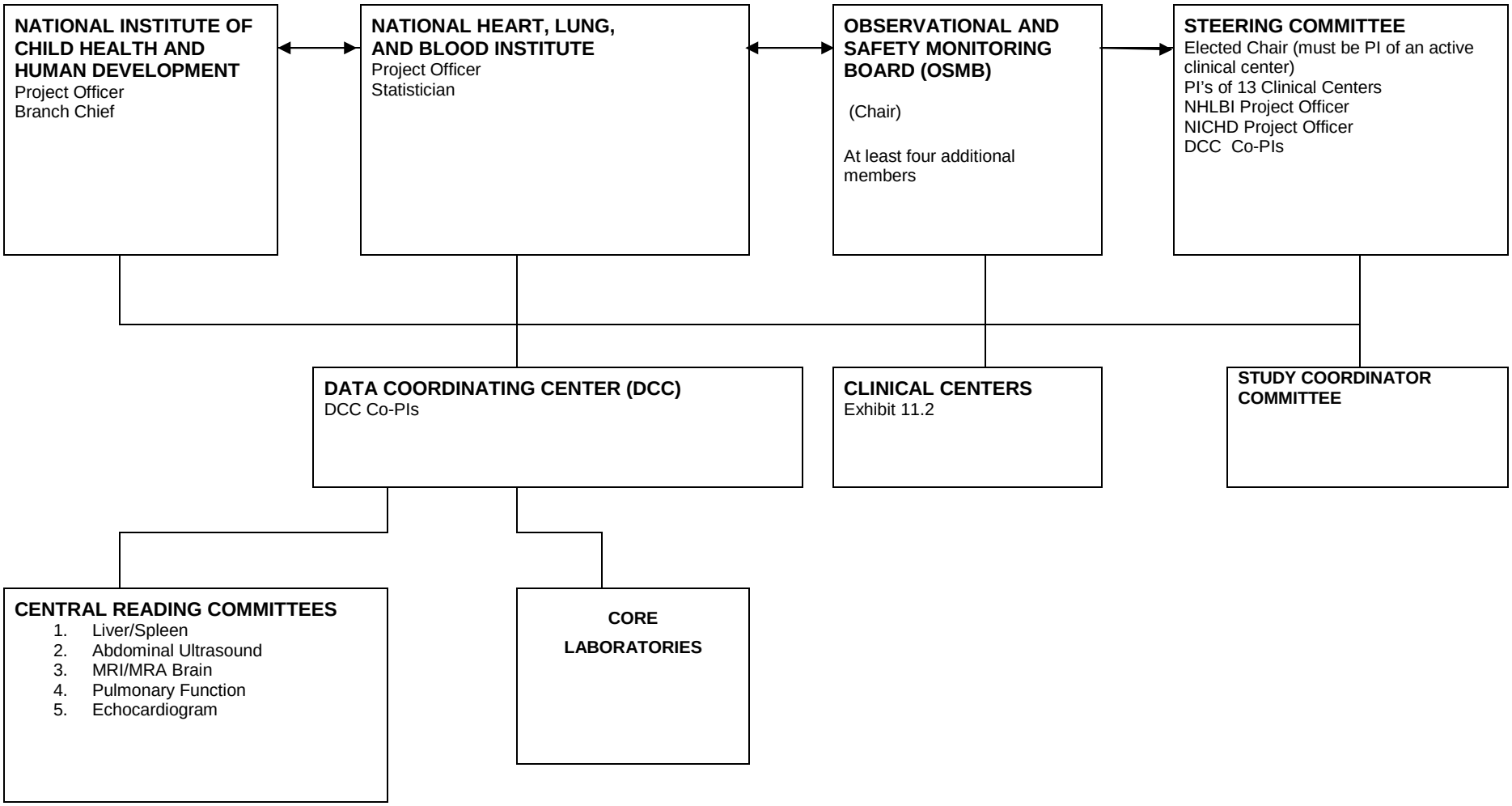

Exhibit 11.2
PARTICIPATING CLINICAL CENTERS

CLINICAL CENTERS

Children's National Medical Center, Naomi Luban, M.D, Brenda Martin RN, CPCP. - 01
(Washington, DC)

Duke University Medical Center, Jennifer Rothman, M.D. - 02
(Durham, NC)

Howard University College of Medicine, Sohail Rana, M.D. - 03
(Washington, DC)

Johns Hopkins University School of Medicine, James F. Casella, M.D., Ph.D. - 04
(Baltimore, MD)

Medical University of South Carolina, Sherron Jackson, M.D. - 05
(Charleston, SC)

St. Jude Children's Research Hospital, Winfred C. Wang, M.D. - 06
(Memphis, TN)

State University of New York - Brooklyn (SUNY), Scott T. Miller, M.D. - 07
(Brooklyn, NY)

University of Miami School of Medicine, Ofelia Alvarez, M.D. - 08
(Miami, FL)

University of Mississippi Medical Center, Suvankar Majumdar, M.D. - 09
(Jackson, Mississippi)

University of Texas Southwestern Medical Center, Zora R. Rogers, M.D. - 10
(Dallas, TX)

University of Alabama, Birmingham, Jeffrey Lebensburger,D.O. - 11
(Birmingham, AL)

Emory University School of Medicine, R. Clark Brown, M.D., Ph.D. - 13
(Atlanta, GA)

Wayne State University, Ingrid Sarnaik, M.D. - 14
(Detroit, MI)

DATA COORDINATING CENTER

New England Research Institutes (Watertown, MA)
Susan Assmann, PhD, Principal Investigator
Julie Miller, MPH, Co-Principal Investigator

PROJECT OFFICE

Division of Blood Diseases and Resources
National Heart, Lung, and Blood Institute (Bethesda, MD)
Ellen M. Werner PhD, MA, Project Officer
Myron Waclawiw, Ph.D., Statistician

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APPENDIX A

Schedule of Blood and Urine Collection and Schedule of Special Studies

BABY HUG Follow-up Observational Study II

Time Point	Clinical Data Reports	Blood Collections									Urine Collection		Other Tests								Physical Exam
	6 Month Data Abstraction Form	HbF **core**	CBC, Retics, Diff **local**	LDH, Billi, ALT **local**	HJB/ Reticulocyte micronuclei **core**	Pitted Cells **core**	Stored Blood Sample **core**	VDJ, DNA Extraction **core**	Cystatin C **core**	Creatine and BUN **core**	Urine Osmo **local**	Microalb: Creat Ratio **core**	Liver/ Spleen **central**	Abdom Sono **central**	TCD **central**	Pulm Func Tests **central /CBC local**	Cardiac Echo **central /BNP local**	MRI/ MRA Brain **central**	Neuro-psych: Vineland, WISC-IV, Connor CPT II, Peds QOL **local**	Questionnaire CRF **local**	
PASSIVE FOLLOW-UP CARE																					
Study Entry	7/1/2012	X	X	X	X	X	X^	X	X	X		X									
Every 6 Months for 60 Months	X																				
Every 12 Months for 60 Months																				X	
When Performed as Part of Clinical Care (not paid as part of the study)													X*	X*	X*	X*	X+*	X*	X*		X*
End of Study	X	X	X	X	X	X	X^	X	X	X		X									
ACTIVE FOLLOW-UP CARE																					
Study Entry	X	X	X	X	X	X	X^	X	X	X	X	X									X
Every 6 Months for 60 months	X																				
Every 12 Months for 60 Months		X	X	X	X	X			X	X										X	X
Once during the study at age 10													X	X	X	X	X+	X	X		
End of Study	X	X	X	X	X	X	X^	X	X	X	X	X									X

^The Stored Blood and Urine samples will be collected, separated and aliquots made and stored for future study. +Brain Natiuretic Peptide (BNP) will be performed locally once during the study at the time of an Echocardiogram. *Results for 'Other Tests' performed in the passive group should be reported to the DCC on the appropriate Case Report Form and images obtained as a result of 'Other Tests' that are performed on subjects enrolled in the passive group should be sent to the DCC for central reading.

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APPENDIX B

Core Laboratory Determinations and Specimen Requirements
BABY HUG Follow-up Observational Study II

Specimen	Volume	Collection tube type	Frequency	
			Active	Passive
Urine for Microalbumin: Creatinine Ratio and Stored Urine Sample	10 mL	Cryovial	Entry, Exit	Entry, Exit
Stored Blood Sample	5.0 mL	EDTA lavender top	Entry, Exit	Entry, Exit
Cystatin C	1.0 mL	Red top	Entry, q12 months, Exit	Entry, Exit
HbF	0.5 mL	EDTA lavender top	Entry, q12 months, Exit	Entry, Exit
Howell Jolly Bodies/ Reticulocyte Micronuceli	1.0 mL	EDTA lavender top	Entry, q12 months, Exit	Entry, Exit
Pitted Cells	0.1 mL	EDTA lavender top w/gluteraldehyde	Entry, q12 months, Exit	Entry, Exit
Creatinine and BUN	1.0 mL 4.0 mL	Red top Red top	Entry, Exit q12 months	Entry, Exit
VDJ/ DNA Extraction/Storage	3.0 mL	EDTA lavender top	Entry, Exit	Entry, Exit